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Running Head: S100B Response to Playing American Football

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ABSTRACT

Objective: To determine if serum S100B increases similarly as a result of playing American football compared to exercise alone. **Methods:** Serum S100B was measured in division III collegiate football players before and after every home game during a single football season. Serum S100B was also measured before and after subjects walked on a treadmill for 30 minutes at a leisurely pace, ran on a treadmill while wearing and not wearing a football helmet at 6 mph for 8 minutes, and performed low, moderate, or high intensity resistance exercise. **Results:** Serum S100B increased significantly ($p < 0.05$) when subjects played in a football game, ran on a treadmill, or performed moderate intensity resistance exercise. Pre-game serum S100B did not accumulate throughout the football season in any of the players ($p > 0.05$). The increase in serum S100B during football games was moderately and significantly correlated to both the number of hits ($R^2 = 0.407$) and plays ($R^2 = 0.484$) that each player experienced ($p < 0.001$). Post-game serum S100B was greater in football players who played more than 50 plays compared to those players who played less than 50 plays, subjects who exercised on a treadmill, or subjects performing resistance exercise ($p < 0.05$). **Conclusion:** It is unclear if the higher S100B concentration in football players playing at least 50 plays was caused by exercise or hits. Therefore, if serum S100B is to be used as a biomarker of impacts, and possible brain injury in sport, exercise time and intensity should be taken into account as confounding variables.

Key Words: Exercise, Subconcussion, Sport-related concussion

Introduction

Increased levels of protein S100B in cerebral spinal fluid (CSF) have been clinically linked to the brain since the late 1970s and early 1980s when Michetti and colleagues first discovered that the S100 antigen in CSF was associated with neurological diseases^{1,2}. Following these findings, data from clinical research has also supported the relationship between brain injury and elevated levels of S100B in CSF and blood serum³⁻¹².

Beginning in the 21st century, S100B has been under investigation as a serum biomarker of mild traumatic brain injury in sport, specifically as an indicator of sport-related concussion and subconcussion injury¹³⁻²⁵. Although serum S100B concentration ([S100B]) has been shown to increase in response to the number of contacts an athlete experiences^{16-18,21,25}, [S100B] has also been shown to rise in relation to exercise alone^{16,26-28}. Findings of increased [S100B] caused by marathon running²⁷, a 25 km running race¹⁶, jogging 10 km¹⁶, running for 40 minutes at ventilatory threshold²⁸, sprinting for two minutes¹⁶, and a 7,600 m swimming race²⁶; paired with the presence of S100B in extracranial tissue²⁹⁻³¹, has cast doubt over the use of S100B as a valid biomarker of brain injury in sport^{20,32}.

The purpose of this study was to determine if the exercise required to play an American football game is a confounding variable that compounds any increase in [S100B] potentially caused by impacts. To compare [S100B] caused by playing an American football game in absence of apparent head injury to exercise alone; [S100B] was measured in American football (football) players, not diagnosed with any brain injury, after four football games and compared to [S100B] after subjects exercised on a treadmill or performed resistance exercise.

Materials and methods

Informed consent was obtained from all subjects, and the University of Wisconsin-Platteville Institutional Review Board for the protection of human subjects (Platteville, WI, USA) approved the study. The overall study design is depicted in figure 1.

Football study design. Sixteen male Division III collegiate football players participated in this study. Prior to the start of training camp, a baseline blood draw was performed on nine of the 16 players. During the season, blood was drawn on the Friday immediately (within 30 minutes) after practice before each Saturday home football game (four games total). The blood draw was performed on Friday to minimize interruption of pre-game rituals on Saturday. Practice on Friday was thought to cause little changes in [S100B] because the practice was always a “walk-through”, where the players would go over plays while wearing a helmet but no other padding. Immediately following each Saturday home game, the subjects returned for a post-game blood draw obtained within 30 minutes after conclusion of the game. No athletes in the subject pool were diagnosed with concussion during any of the home games.

The number of athletes that played each game, and the amount of time they played varied, as determined by the coaching staff. For clarification, the word “play” throughout this paper refers to the act of playing in a football game and is not equivalent to the number of games played. In games two and four, one player forgot to return for the post-game blood draw. Data from a player in game two was removed because he had suffered a concussion the week prior (at an away game) and had a pre-game [S100B] twice greater than his normal levels. Five athletes played in every game of the season (both home and away), and the data from these athletes were used to determine if [S100B] accumulated over the season. Game film recordings were visually analyzed after each game by the same investigator who was an 8th grade football coach and trained varsity high school football referee. This investigator analyzed the film to determine the number of plays (anytime an athlete was on the field from the snap of the ball to begin play, until the blow of the whistle to end play) each athlete participated in and the number of hits (anytime two players contacted each other as a result of blocking, tackling, or incidental contact) each athlete experienced. Hits were subjectively categorized by the trained investigator into category I and category II hits. Category I hits were considered to be typical of a football game (normal tackling and blocking), whereas category II hits were considered to be of greater magnitude than routinely experienced in a game; such as would be experienced when a

player is not expecting to be blocked or tackled. Category II hits also caused a noticeable jarring of the head and body, which was not present with category I hits.

Treadmill protocols. A group of college-aged males and females were recruited to participate in various treadmill protocols. The walking protocol required six subjects to walk at a self-determined leisurely pace for 30 minutes. This protocol was done to determine if light exercise has any impact on [S100B].

In an additional treadmill protocol, eight subjects participated in treadmill running for eight minutes in duration at a speed of six miles per hour (mph). Eight minutes were chosen to overcompensate for the amount of time any of the athletes played in one football game. For example, the most plays a football player participated in was 83 plays, and each play lasted approximately 5 seconds as determined by watching game-day film; therefore, we can estimate this player being involved in 6.9 minutes of actual play. The speed of six mph was chosen to increase the intensity well above walking, but still of low enough intensity to allow the recreationally active subjects to complete the eight-minute protocol. This protocol was conducted for each subject on two separate occasions in a counterbalanced order; one time while wearing a football helmet and one time while not wearing a football helmet. Requiring running with and without a helmet was necessary to determine if simply wearing a helmet causes a greater increase in [S100B]. Blood was drawn immediately before and after (within 30 minutes) each treadmill protocol.

Resistance protocol. Eighteen college-aged subjects participated in the resistance exercise protocol. Since skeletal muscle has been shown to produce S100B²⁹, the purpose of the resistance exercise protocol was to examine increasing levels of [S100B] as intensity, and likely muscle damage, increased. The resistance protocol required subjects to undergo either a low, moderate, or high intensity workout based on each individual subject's one-repetition maximum (1RM) for the following free weight exercises: dumbbell squat, dumbbell flat bench press, dumbbell bicep curl to shoulder press, leg press, and kneeling overhead triceps extension. Each subject's 1RM was

determined for each exercise by gradually increasing the resistance for each exercise until the subject was no longer able to perform one repetition with correct form for that particular exercise. The last resistance at which the subject was able to complete one repetition with correct form was considered the subject's 1RM for that exercise.

Participants were then placed into one of three different groups: low intensity (30% 1RM), moderate intensity (60% 1RM), or high intensity (90% 1RM). All three groups completed the same workout in the same order but with a resistance corresponding to the different percentages of their 1RM. Each exercise was performed for 30 seconds followed by 30 seconds of rest, with this cycle being repeated for four sets (two minutes of exercise and two minutes of rest). After four sets, the subject was given one minute of rest to transition to the next exercise. Blood was drawn immediately before and after (within 30 minutes) each resistance protocol. (insert figure 1 here)

Blood collection and processing. Blood was drawn from a prominent vein in the antecubital space into one 4 ml serum separation vacutainer. Following blood collection, each sample was allowed to coagulate at room temperature for at least 30 minutes but no greater than 60 minutes. At the end of the incubation period the blood samples underwent centrifugation at 3,500 RPM for 10 minutes at 4°C. Serum was then drawn from the separated blood sample and frozen at -80°C until analysis of [S100B].

Biochemical analysis. Serum [S-100B] was measured using a Human S100B ELISA-96-well plate assay kit (EZHS100B-33K, EMD Millipore Corporation, Billerica, MA) with absorbance read using a UV-Vis microplate reader (accuSkan Go, Thermo Fisher Scientific, Inc., Waltham, MA USA). The range of the assay was 0.0027 – 2 $\mu\text{g}\cdot\text{L}^{-1}$. Analysis of standards and samples were all performed in duplicate. A five-parametric logistic regression analysis was used to formulate a standard curve for each assay (average $R^2 = 0.99978$) using KaliedaGraph version 4.5 (Synergy Software, Reading, PA USA). The average intra-assay coefficient of variation was 5.04% while the inter-assay coefficient of variation was an average of 7.74%.

Statistical analysis. For all data, the assumption of normality was determined using a Shapiro-Wilk test, while homogeneity of variance was determined using a Levene's test. A 95% confidence interval (CI) was used to assign bounds of expected discrepancy between sample and population means. A Cohen's d (d) test was performed to determine effect size when paired data were being analyzed³³. Partial eta squared (η^2) was used to calculate effect size when a Mann-Whitney U or Kruskal-Wallis test was performed. Dunn-Bonferroni post hoc analyses were used when significant differences were present among protocols. Alpha was set at 0.05 to determine statistical significance. All statistical analyses were generated using SPSS version 24 (SPSS Inc., Chicago, IL USA).

Pre- vs. post-protocol [S100B]. A non-parametric two-tailed Wilcoxon Rank Sum test was performed to compare mean pre- and post-protocol [S100B] in football players who played in each game and subjects running on a treadmill without wearing a football helmet since the assumption of normal distribution required to perform a parametric paired T-test was not met. A paired two-tailed T-test was used to compare mean pre- and post-protocol [S100B] in football players who did not play in each game, as well as for the protocols of: running on a treadmill while wearing a football helmet, treadmill walking, and low, moderate, and high intensity resistance exercise since all assumptions to perform a parametric T-test were met for these protocols.

Correlation data. A Spearman Rho correlation was used to compare number of level I hits and number of plays to post-game [S100B]. The correlation between number of plays and number of hits was also analyzed using a Spearman Rho correlation. The non-parametric Spearman Rho was used for correlation analyses since the assumptions of normality and homoscedasticity were not met for the use of a parametric Pearson correlation.

Effect of hit category on [S100B]. Post-game [S100B] in football players who experienced category I and II hits (no player solely experienced category II hits) were compared to those who experienced only category I hits or zero hits in order to determine if the category of hit experienced by the football players resulted in differences of [S100B]. A non-parametric Kruskal-Wallis test was used to make this comparison as the data did not meet the assumptions of normality or

homogeneity of variance to perform a parametric Univariate ANOVA analysis. Post-game [S100B] was also compared between the players who experienced at least 50 plays receiving only category I hits to post-game [S100B] in players who experienced both category I and II hits using a Mann-Whitney U test since the assumption of normality was not met to conduct a parametric unpaired T-test. This comparison was done to determine if category II hits caused greater post-game [S100B] than could be achieved by playing at least 50 plays and experiencing only category I hits.

[S100B] comparison across protocols. Prior to comparing [S100B] among protocols, we wanted to confirm that the Friday “walk-through” practice did not affect [S100B]. To do this, pre-game [S100B] for games 1, 2, 3, and 4 were compared to each other, to pre-training camp values, and to the pre-protocol values for the treadmill and resistance protocols using a Kruskal-Wallis test since the assumption of normality was not met to perform a parametric Univariate ANOVA.

For comparison across protocols, the football data was categorized into three groups based off the number of plays the athletes experienced. Players who were in the football game for zero plays were put into one category (0 plays), players who were in the football game for less than 50 plays were placed into a second category (<50 plays), and players who were in the football game for 50 or more plays were placed into a third category (≥ 50 plays). “Fifty plays” was chosen as a cut-off point because there was a significant difference in [S100B] when athletes were involved in at least 50 plays compared to when the athletes were involved in less than 50 plays. With this categorization, all data from athletes who experienced category II hits in a game (13 players over the four games) were removed. This manipulation of the data was done to better compare post-game [S100B] caused by “normal” football play, to post-exercise [S100B] caused by the treadmill and resistance protocols. Mean post-protocol [S100B], was compared among all protocols using a Kruskal-Wallis test. The non-parametric Kruskal-Wallis test was used to make comparisons among [S100B] for the protocols since the assumptions of normality and homogeneity of variance were not met to perform the parametric Univariate ANOVA.

Results

Descriptive statistics of the data used for analysis among all protocols can be seen in table 1.

Figure 2 illustrates pre- vs. post-protocol [S100B]. Pre-training camp data for the football players is not included in Figure 2. In football games, [S100B] increased significantly from pre- to post-game for athletes who played in the game ($p < 0.001$, $d = 0.410$), but decreased significantly in athletes that did not receive any playing time ($p = 0.042$, $d = 0.453$). Treadmill exercise at 8 mph for six minutes caused a significant increase in [S100B] in both wearing a football helmet ($p = 0.001$, $d = 1.15$) and not wearing a football helmet ($p = 0.012$, $d = 0.653$). Neither treadmill walking ($p = 0.707$, $d = 0.075$), low resistance exercise ($p = 0.348$, $d = 0.283$), nor high resistance exercise ($p = 0.308$, $d = 0.362$) caused a significant increase in [S100B]. Moderate resistance exercise ($p = 0.029$, $d = 0.548$) did cause a significant increase in [S100B]. (insert Figure 2 here)

As shown in figure 3, pre-game [S100B] did not increase as the season progressed in the athletes who played every game of the season ($p = 0.770$, $\eta^2 = 0.026$). Figure 4 shows that post-game [S100B] was moderately and significantly correlated to both the number of level I hits that athletes received ($R^2 = 0.407$, $p < 0.001$), and the number of plays that athletes were involved in ($R^2 = 0.484$, $p < 0.001$). The correlation between the number of plays and number of level I hits a football player experienced (data not shown) was found to be high ($R^2 = 0.893$, $p < 0.001$). (insert figures 3 and 4 here)

Figure 5 shows that pre-game [S100B] was significantly greater before games 1, 2, and 3 compared to pre-training camp and pre-game 4 [S100B] ($p < 0.05$, $\eta^2 = 0.402$), while pre-game [S100B] before game 2 was greater compared to all other pre-exercise scenarios except treadmill walking ($p < 0.05$, $\eta^2 = 0.402$). This comparison shows that the Friday “walk-through” practice caused significant changes in pre-game [S100B]. The significant decrease in [S100B] from the Friday walk-through to the end of the game in football players who practiced but did not play in the game (figure 2) further confirms that even the pre-game walk-through can cause an elevation of [S100B]. Due to these findings, post-protocol [S100B] was used for comparison across protocols. (Insert figure 5 here)

When comparing how the category of hit effected [S100B] (figure 6) it was found that category II hits caused a significant increase in post-game [S100B] compared to both category I and no hits. ($p < 0.05$, $\eta^2 = 0.412$). Interestingly, there was no significant difference in post-game [S100B] between players experiencing category I hits and those who experienced no hits ($P > 0.05$, $\eta^2 = 0.412$). Comparison of [S100B] in football players involved in at least 50 plays and only category I hits to those involved in both category I and II hits (data not illustrated) showed that post-game [S100B] was not statistically different between the two groups ($p = 0.096$, 0.145). In figure 7, post-game [S100B] for football players were separated by the number of plays athletes participated in. Athletes who experienced any category II hits were not included in the figure 7 data set for better comparison across all protocols, and it was found that post-game [S100B] was significantly greater for athletes who participated in 50 or more plays compared to all other protocols ($p < 0.05$, $\eta^2 = 0.203$). (insert figures 6 and 7 here)

DISCUSSION

Football protocol. The results of this study indicate that playing a collegiate division III American football game causes a significant increase in [S100B], and is in agreement with our previous research on junior varsity collegiate division III players¹⁹. Pre-game [S100B] did not accumulate throughout the season for the five athletes who played in every single game. This has been shown previously in football players²¹ and indicates that [S100B] is not a good indicator of hit accumulation throughout a season.

Post-game [S100B] was moderately, and significantly, correlated to the number of category I hits athletes were exposed to during each game. A relationship between hits and [S100B] has been previously documented in football players²³. Unique to this study was the comparison of the number of plays an athlete was involved in to [S100B]. This comparison showed that increases in [S100B] were also moderately and significantly correlated to the number of plays an athlete was involved in; indicating that exercise may result in similar increases of [S100B] to that caused by hits.

Past research has also indicated that [S100B] may increase independently of head injury, as illustrated by increases in [S100B] following participation in exercise where head injury is uncommon³². Findings include increased [S100B] as a result of: swimming²⁶, running^{16,27,28}, and number of jumps while playing basketball¹⁵. Playing soccer has also been shown to cause increases in [S100B], of which the cause has reasonably been attributed to the number of head contacts with the ball (headers) a player experiences^{13,14,34}. However, [S100B] has not been shown to change as a result of heading soccer balls independent of exercise^{16,24,34–36}.

Comparison of [S100B] among hit categories and across protocols. The category of hit received by the football players affected post-game [S100B] significantly; with players who experienced both category I and II hits (no player solely experienced category II hits) having the highest post-game [S100B] compared to players who experienced only category I hits or no hits. This was unexpected since category II hits did not cause concussion or any outward signs of brain damage as compared to category I hits. Even more unexpected was the finding of no significant difference between post-game [S100B] in players only experiencing category I hits and those experiencing no hits. This was unexpected because all players who did not experience hits did not receive any playing time during the game except one player who played in one play as a long-snapper (snapped the ball to the kicker) and did not receive a hit.

In order to determine if exercise alone resulted in similar increases of [S100B] as playing in a football game, treadmill and resistance exercise protocols were included in this study. To better compare [S100B] across the different protocols, data from the football players who experience category II hits (13 players over 4 games) were removed so that [S100B] caused by “normal” football play could be compared. With this comparison, it was found that athletes who played in 50 or more plays had a significantly greater post-game [S100B] compared to those athletes who did not play, those who played 1-49 plays, and the treadmill and resistance protocols. Athletes who did not play, or who played in 1-49 plays, did not have a significantly different post-game [S100B] compared to each other or compared to the treadmill and resistance exercise protocols. Interestingly, when the post-game [S100B] data from the players who were involved in at least 50 plays but no category II hits

were compared to the post-game [S100B] data from the players who experienced both category I and II hits, no statistical difference between the two groups was found.

These findings imply two separate conclusions: 1. Playing football causes elevated [S100B] as plays and hits accumulate, possibly indicating greater damage to the brain compared to non-contact exercise, or 2. The greater mean [S100B] found in football players ($0.077 \mu\text{g}\cdot\text{L}^{-1}$) indicates that playing football is a higher intensity exercise than the treadmill ($0.028 \mu\text{g}\cdot\text{L}^{-1}$) or resistance ($0.022 \mu\text{g}\cdot\text{L}^{-1}$) protocols used in this study. Exercise intensity may play a greater role in [S100B] than exercise duration since total playing time in the football games (under 7 minutes) were less than the treadmill running (8 minutes) and walking times (30 minutes). Past studies have also indicated that exercise intensity may have a greater influence on [S100B] than duration since playing soccer (typically 55 minutes of play) ($0.18 \mu\text{g}\cdot\text{L}^{-1}$)¹³ and basketball (typically 40 minutes of play) ($0.299 \mu\text{g}\cdot\text{L}^{-1}$)¹⁵ have both resulted in higher post-game [S100B] compared to marathon running (median finish time = 247 minutes) ($0.16 \mu\text{g}\cdot\text{L}^{-1}$)²⁷ or a 7,600 m swim race (mean finish time = 107 minutes) ($0.108 \mu\text{g}\cdot\text{L}^{-1}$)²⁶.

Conclusion. The main focus of this study was to determine if sports-related exertion has a compounding impact on [S100B] that would make the use of [S100B] as an indicator of brain injury in sport more ambiguous compared to the clinical success that this protein has had in indicating brain injury. Our results suggest that playing football increases [S100B] to a greater extent than non-contact exercise, possibly because playing football is of greater exercise intensity compared to the treadmill and resistance exercise protocols used in this study. When exercise time and intensity are not controlled, the increase in [S100B] is highly variable as shown by the high standard deviations and 95% confidence intervals found in the football players compared to those found in the treadmill and resistance protocols, where exercise time and intensity were controlled. Due to these findings, we conclude that it may be impractical to use [S100B] as an indicator of sports-related brain injury since exercise time and intensity are highly variable among athletes and virtually impossible to monitor or

control in game situations without special equipment. The ideal blood biomarker should not be influenced by exercise duration or intensity if it is to be used as an indicator of brain injury in sport.

Limitations. There are several limitations to the interpretation of these data. First, the number of subjects involved in the study were quite low in each protocol. A larger sample size of football players, as well as subjects participating in the resistance and treadmill protocols would improve the interpretation of the results. Although the time of day for each blood draw was similar in the football players, it varied widely in the subjects participating in the treadmill and resistance exercise protocols. This may have caused changes in [S100B] as a result of diurnal rhythm; however, there are no studies to date that have investigated if [S100B] is affected by diurnal rhythm. The lack of data from concussed subjects was also a limitation to this study. It is possible that [S100B] responds in greater magnitude when brain injury is present compared to exercise alone.

Lack of objective assessment of exercise duration and intensity along with number and magnitude of hits during football games was also a limitation to this study and should be improved in future studies by using technological instrumentation to analyze these variables. Using trained and untrained subjects may have skewed the results of the resistance exercise protocols. The main purpose of including resistance exercise was to elicit muscle damage, and the subsequent release of S100B from skeletal muscle. Since some subjects were trained in resistance exercise, the protocols may not have induced the same muscle damage in trained compared to untrained subjects.

Perspective

Since the year 2000, serum protein S100B has been investigated as a potential biomarker of brain injury in sport. Results from research in contact sports looked promising, as [S100B] increased in correlation with hits to the head. However, research investigating [S100B] in response to exercise alone also resulted in elevated S100B levels. Our study is the first to investigate the relationship between athlete playing time and number of hits as a result of playing American football, showing similar correlation of both to [S100B]. We also compared the rise in serum S100B caused by playing American football to that caused by walking, running, and resistance exercise. Based off

our observations, exercise amplifies any increases in serum S100B that may result from impacts in football players. Therefore, if protein S100B is to be used as an indicator of impacts in sport, exercise duration and intensity must be taken into consideration as confounding variables.

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Figure legends

Figure 1: Flow chart depicting the study design for the football protocol, treadmill protocol, and resistance exercise protocol. For details please see the materials and methods section.

Figure 2: Comparison of pre-serum [S100B] compared to post-serum [S100B] for all protocols. Pre-game and post-game football data from all four games were combined for those athletes who played in a game and those who did not play in a game. Pre-training camp data were not included for the football players in this figure. Played (n=36), did not play (DNP) (n=25), treadmill walking (n=6), treadmill running without (w/out) helmet (n=8), treadmill running with helmet (w/helmet) (n=8), low intensity resistance exercise (n=6), moderate intensity resistance exercise (n=6), high intensity resistance exercise (n=6), * = significantly different compared to pre-serum [S100B] ($p < 0.05$).

Figure 3: Pre-game serum [S100B] for all four games in the athletes who played every game of the season, both home and away (n = 5).

Figure 4: Spearman rho correlation between number of hits and post serum [S100B] as well as between number of plays and post serum [S100B] (n = 61). Note: the two outliers on the right side of the graph represent the same subject.

Figure 5: Baseline serum [S100B] across all protocols. Pre-training camp (n=9), pre-game 1 (n=16), pre-game 2 (n=15), pre-game 3 (n=16), pre-game 4 (n=16), pre-treadmill walking (n=6), pre-treadmill running with helmet (w/helmet) (n=8), pre-treadmill running without (w/out) helmet (n=8), pre-low intensity resistance exercise (n=6), pre-moderate intensity resistance exercise (n=6), pre-high intensity resistance exercise (n=6); a = significantly different compared to pre-game 2 ($p < 0.05$); b = significantly different compared to pre-training camp ($p < 0.05$); c = significantly different compared to pre-game 4 ($p < 0.05$).

Figure 6: Post-game [S100B] among athletes who experienced zero hits, those who only experienced category I hits, and those who experienced both category I and II hits. * = significantly greater than category I hits and zero hits

Figure 7: Post-serum [S100B] across protocols with the football players separated into three different participation categories. Data from players experiencing category II hits was removed for this comparison. Zero (0) plays (n = 25), less than 50 (<50) plays (n = 17), greater than or equal to 50 (≥ 50) plays (n = 5), treadmill walking (n = 6), treadmill running with helmet (w/helmet) (n = 8), treadmill running without (w/out) helmet (n = 8), low intensity resistance exercise (n = 6), moderate intensity resistance exercise (n = 6), high intensity resistance exercise (n = 6). * = significantly greater post serum [S100B] compared to all other scenarios.

TABLE 1. Descriptive statistics for all protocols. Subjects range from ages 18-22 yrs. All subjects were Caucasian with the exception of one African American male subject in the football and treadmill running protocols. S100B is reported as $\mu\text{g}\cdot\text{L}^{-1}$.

	Pre-[S100B]	Post-[S100B]
<u>Football Games (all subjects were male)</u>		
Pre-training camp (n = 9)	0.020±0.005 (0.016-0.024)	Not Applicable
Played (n=36)	0.046±0.053 (0.028-0.064)	0.077±0.093 (0.045-0.108)
Did Not Play (n=25)	0.032±0.010 (0.027-0.036)	0.027±0.012 (0.022-0.032)
0 plays (n=25)	Not used in analysis	0.027±0.012 (0.022-0.032)
1-49 plays (n=17)	Not used in analysis	0.045±0.061 (0.014-0.076)
≥50 plays (n=5)	Not used in analysis	0.052±0.011 (0.039-0.067)
0 hits (n=26)	Not used in analysis	0.027±0.012 (0.022-0.032)
Category I hits only (n=21)	Not used in analysis	0.047±0.54 (0.023-0.072)
Category I & II hits (n=14)	Not used in analysis	0.124±0.121 (0.054-0.194)
Game 1 P (n=8)	0.043±0.049 (0.002-0.084)	0.067±0.053 (0.023-0.111)
Game 1 DNP (n=8)	0.028±0.006 (0.022-0.033)	0.020±0.006 (0.015-0.025)
Game 2 P (n=6)	0.078±0.068 (0.007-0.150)	0.169±0.160 (0.001-0.336)
Game 2 DNP (n=8)	0.041±0.006 (0.036-0.046)	0.035±0.011 (0.026-0.043)
Game 3 P (n=10)	0.046±0.051 (0.009-0.083)	0.058±0.061 (0.014-0.102)
Game 3 DNP (n=6)	0.032±0.011 (0.020-0.043)	0.028±0.013 (0.014-0.041)
Game 4 P (n=12)	0.033±0.048 (0.002-0.064)	0.052±0.072 (0.006-0.098)
Game 4 DNP (n=3)	0.019±0.007 (0.001-0.037)	0.020±0.015 (0.000-0.058)
<u>Football Players Who Played Every Game of the Season</u>		
Game 1 (n=5)	0.055±0.061 (0.000-0.1302)	Not used in analysis
Game 2 (n=5)	0.079±0.076 (0.000-0.173)	Not used in analysis
Game 3 (n=5)	0.069±0.068 (0.000-0.153)	Not used in analysis
Game 4 (n=5)	0.054±0.073 (0.000-0.144)	Not used in analysis
<u>Treadmill Protocols (3 subjects in each protocol were female)</u>		

Walking (n=6)	0.026±0.010 (0.016-0.036)	0.025±0.016 (0.008-0.042)
Running H (n=8)	0.022±0.005 (0.018-0.026)	0.029±0.007 (0.023-0.035)
Running NH (n=8)	0.023±0.005 (0.019-0.026)	0.029±0.012 (0.019-0.038)
<u>Resistance Exercise (2 subjects in Low and Mod were female; 4 subjects in High were female)</u>		
Low (n=6)	0.014±0.012 (0.001-0.026)	0.017±0.009 (0.007-0.026)
Mod (n=6)	0.017±0.015 (0.001-0.033)	0.027±0.021 (0.005-0.049)
High (n=6)	0.017±0.015 (0.001-0.032)	0.023±0.018 (0.005-0.042)
<u>Differences in Race (Protein S100B has been found to be enriched in melanocytes³¹)</u>		
Caucasian (n=46)	0.033±0.035 (0.026-0.040)	0.045±0.062 (0.032-0.057)
African American (n=2)	0.017±0.006 (0.009-.024)	0.025±0.010 (0.013-0.037)

Values are represented as mean±SD (95% Confidence Interval)

Played (P), Did not play (DNP), Helmet (H), No helmet (NH), Low resistance (Low), Moderate resistance (Mod), High resistance (High)







