

Minor Head Trauma in Soccer and Serum Levels of S100B

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Abstract

Objective: To compare the serum levels of S100B after a head trauma to the effect of heading, high-intensity exercise and playing a league match. Heading and head traumas in soccer have been suspected to cause brain impairment. The protein S100B is a marker of acute neuronal tissue damage.

Method: Baseline S100B was measured in 535 Norwegian professional soccer players. 228 head impacts were registered from 352 league matches. Three teams (N=48) performed a high-intensive exercise session without heading and a low-intensity session with heading exercises. Blood samples were drawn within one hour (B1) and the following morning (B12) after a match/training for the four groups: Head Impact (N=65), Match Control (Match participants without head impact, N=49), High-Intensive Exercise (N=35), Heading (N=36).

Results: Serum S100B increased from baseline to B1 for all groups. The increase for the match groups (Head Impact and Match Control) was significantly higher than for both the training groups. However, no significant differences between the Head Impact and Match Control groups or between the two training groups were found. A total of 39 (33.9%) players showed elevated B1 values (≥ 0.12 ng/mL) after a match, but these were equally distributed between the Match Control Group and the Head Impact Group.

Conclusion: Both **soccer training and soccer** matches cause a transient increase in S100B. There is a possible additive effect of activity with high intensity and heading, but minor head impacts do not seem to cause an additional increase.

Running Title: MHT in Soccer and S100B

Keywords: Soccer [MeSH]; closed head trauma [MeSH]; Brain injury [MeSH]; S100 Proteins [MeSH]

Introduction

Soccer is one of the few sports where an unprotected head is used actively for heading and advancing the ball (30). When heading was introduced in soccer, this feature was first looked upon as ludicrous and “not soccer”, but later it has developed to become an important part of defensive and offensive play (49). However, during the last two decades there has been an increasing concern that heading could lead to chronic brain injury as seen in boxing. This was first postulated by Tysvaer in 1992 (65) based on a series of cross-sectional studies using neurological exams, neuropsychological tests, computer tomography (CT) scans and electroencephalography (EEG) exams on active and older retired Norwegian soccer players. Since then, some cross-sectional studies have indicated that soccer can cause measurable cognitive impairment (19, 33-35), while others have not detected such a relationship (23, 60). Heading duels also expose the players to an increased risk of sustaining a head trauma (2, 60), and it has been hypothesized that the reported cognitive deficits are more likely to be the result of accidental head impacts that occur during the course of the matches rather than heading (29).

Among injuries related to soccer, 6-13% are head injuries (3, 22). The reported incidence of head injuries for men during matches is 1.7 - 3.5 per 1000 player hours (2, 22). This incorporates all types of head injuries including facial fractures, contusions, lacerations, and eye injuries, while the estimated incidence of concussion is 0.3-0.5 per 1000 match hours (2, 13, 22, 45). However, the rate of brain injuries is difficult to assess (15), and the reported incidences are likely to represent minimum estimates. Andersen et al. (2) identified 192 head impacts on video recordings from elite soccer matches (18.8 per 1000 hours), but only five of these were reported as concussions. A study by Delaney et al. (15) revealed that only one out of five concussions are recognised by the players after a head impact in a match, indicating that many players continue to play with undiagnosed concussions.

Several different markers for brain injury have been investigated during recent years. Based on these, Ingebrigtsen & Romner (26) have concluded that the S100B protein is currently the most promising marker for evaluation of traumatic brain injury in patients with minor head injury. Protein S100B is a Ca^{2+} -binding protein mainly attached to the membranes in glial cells in the central and peripheral nervous system (astrocytes or Schwann cells), although it is also expressed in melanocytes, adipocytes and chondrocytes outside the nervous system (18, 62, 68). The serum levels of S100B increases rapidly after a traumatic brain injury and some studies have reported a 10-15 fold increase above baseline levels, followed by a significant decrease the next 4-6 hours due to its short half-life (10, 27, 28, 38, 48, 63). An increased level of S100B after minor head traumas has been reported to be associated with pathological findings on CT scans (9, 36), prolonged in-hospital stays (38), prolonged absence from work (59), post concussive complaints (14, 50) and disability one year after the incident (53). In addition, S100B is associated with the Glasgow Coma Scale score at admission and the outcome after more severe head injury (46, 64). Nevertheless, the specificity of S100B to brain injury has been questioned (4, 17, 32, 40, 43, 57, 66). Highly increased values have been reported after multi-trauma and burns without head injury (5), as well as smaller increases after swimming (16), running and boxing (16, 42). Yet, the increase seen in S100B concentration after exercise was lower than values reported after minor head traumas (9, 12, 14, 27, 36, 41, 48, 53).

S100B is increased after playing a soccer match and appears to be related to the number of headers (54, 56). However, no large-scale prospective study has assessed S100B levels after minor head impacts in soccer. Thus, this study was designed to assess whether minor head impact in soccer could cause injury to the nervous tissue, measured as an increase in the serum S100B concentration. In addition, we wanted to assess the specific effect of high-

intensity exercise and heading on the serum concentration of S100B to control for these factors.

Methods

Study Design

This is a prospective study in a cohort of professional soccer players, where the serum level of S100B was compared between four different conditions: 1) after a head impact occurring during a regular league match (Head Impact Group), 2) after a regular league match with no recorded head trauma (Match Control Group), 3) after a high-intensity training session without heading (High-Intensity Exercise Group), and 4) after a low-intensity training session with heading exercises only (Heading Group). The blood sampling protocol included in each case a baseline sample (before the season or before the training session), a follow-up blood sample taken right after the match/training session and an additional sample the following morning.

Participants and Test Procedures

All players in the Norwegian elite soccer league, Tippeligaen, were asked to participate in the study prior to the 2004 and 2005 seasons. Tippeligaen comprises 14 teams, each with 23-28 players on an A-squad contract, yielding a total of 320-390 players each season. Written informed consent was obtained from all participants, and the study design was approved by the Regional Committee for Medical Research Ethics, Helse Sør, and the Data Inspectorate.

A total of 289 players consented to participate in the study in 2004 and 332 players in 2005. Thus, the study covered 621 player seasons (161 of these players were included in both seasons). Baseline morning blood sampling prior to both seasons was performed for all teams but one during their preseason training camp at the training centre of the Football Association of Norway (NFF) at La Manga, Spain, in February or March. The final team was tested at their local training facilities in Norway during the same time period. In addition, baseline blood sampling was performed in a subgroup of players (N=49) on three different days during

their two-week training camp to assess the variation in baseline serum S100B concentration.

All baseline samples were taken before training between 7:30 and 10:00 am.

Match Study

During both seasons, all regular league matches were observed live by medical personnel present at the venue and they were asked to record all head impacts during the match. The personnel were either the team's own medical staff covering the match or other local medical personnel recruited by the study administrators. The criteria for including head impacts (Head Impact Group) in the sample were: All situations where; 1) a player appeared to receive an impact to the head (including the face and the neck), 2) the match was interrupted by the referee, and, 3) the player laid down on the pitch for more than 15 s (3).

In any case of a head impact (irrespective of whether or not the player was removed from play), the medical personnel were instructed to draw a blood sample from the player straight after the match, preferably within 1 hour after the trauma (B1), as well as a sample the following morning (within 12 hours after the match, B12). Video recordings of all matches were provided by the Norwegian Broadcasting Corporation (NRK) and reviewed the following morning by one of the authors (TMSN) or a research assistant. When a head impact was identified, the respective team's medical personnel were contacted by phone to check on the follow-up status and, if necessary, to arrange for B12 blood sampling. A control group of players from six of the teams included in the study was recruited to give blood samples within one (B1) and 12 hours (B12) after a regular match where they had not experienced any head trauma (Match Control Group). These six control matches were reviewed on video to verify that no head impacts had occurred to these players and a count was made of the number of headers and other head accelerating events per player (i.e. falls or collisions that did not qualify as head impacts).

In order to check how many of the head impact that resulted in actual time-loss injuries (21), the impacts were also cross-referenced with the injuries reported by the team's medical staff through the injury surveillance system (TISS) administrated by Oslo Sports Trauma Research Centre. This register receives data from all the teams in Tippeligaen, and includes all injuries from all team activities that have resulted in absence from training or match (time-loss injuries), as well as the time and date of the injury, type of match, diagnosis, and the number of days before the player returned to training or match (3). The study protocol also included neuropsychological testing of the players the day after the head impact/control match. These results are described in detail in a separate report (61).

Training Study

Moreover, three of the included teams were asked to participate in two separate training sessions prior to the 2006 season (N=48); one high-intensity soccer training session where heading of the ball was not allowed (High-Intensity Exercise Group) and one low-intensity training session with heading exercises (Heading Group). These sessions were planned in cooperation with the team coach and led by the regular coaching staff. The high-intensity soccer play and heading exercise were organized to be as close to the match situation as possible in terms of the level of intensity, or the number and force of the headers. Normal values for the number of headers per player per match was established by counting all headers in matches that were followed live by one of the study administrators during the 2005 season (N=241 players, 2-4 matches counted per player). The mean number of headers per player was 5.7 (95% CI: 0 to 14.8) per match with large variations between the different playing positions, ranging from 2.8 (95% CI: 0 to 6.9) for the midfielders to 9.6 (95% CI: 3.9 to 20.7) for the central defenders. Goalkeepers practically never headed the ball (0.04 [95% CI: 0 to 0.5]). Thus, no standard number of headers was set for the heading exercise session. However, each player was asked to fill in a questionnaire after both sessions (High-Intensity Exercise

and Heading) assessing their level of fatigue and how often they headed the ball during the current day's training compared to a regular match (much less, less, a little less, same, a little more, more or much more). This score was dichotomized to "less" and "same or more" in the analyses. From video recordings of the heading sessions, one or two different players were selected for each of the drills performed and the number and force (i.e. light, moderate or hard) of the headers were counted. The number of headers for each drill was then summarized to create an estimate of the mean number of headers per player per training session. New baseline morning samples were drawn before the first training session, and subsequently within one hour (B1) and the following morning (B12) after each of the two sessions. The training sessions were arranged on separate days and lasted for 90 minutes excluding warm up, and no other training was done in-between the two follow-up blood samples.

S100B Assay

Venous blood samples were collected from an antecubital vein and drawn into a standard gel 7 mL tube (BD Vacutainer® Blood Collection Tube, New Jersey, USA) and allowed to clot for 30 min before centrifugation (3000g) for 10 min. The resulting serum was divided into two 1.5 mL Eppendorf tubes and frozen within two hours. Serum S100B concentrations were measured using an electro-chemiluminescence assay (ROCHE Elecsys®, ROCHE Diagnostics, F. Hoffmann-La Roche Ltd, Basel, Switzerland). The lower detection limit of the assay is 0.005 ng/mL (ROCHE Elecsys® product information). All analyses were performed at the Department of Clinical Chemistry and Clinical Biochemistry, University of Munich, Germany according to the procedure described by Mussack et al. (40) and Bieberthaler et al. (9). Based on previous studies on S100B after minor head trauma, (9, 11, 12, 26, 38), a cut-off value of 0.12 ng/mL was used to classify the B1 samples as elevated or within the normal range.

Statistics

All blood sample data were log transformed to meet the criteria for normal distribution. The reproducibility for the measurement of the baseline concentration of S100B was assessed using ANOVA for repeated measurements. The square root of the residual mean square was divided by the joint mean of all three measurement points to create a coefficient of variation (CV).

The main effect variables for the study were the serum concentration of S100B at B1 and B12, the Delta B1 values (change from baseline to post impact/match/training), and the proportion of players within each group with an elevated B1 sample value. The null hypothesis that there was no difference between groups in S100B serum concentration was tested using repeated measurements ANOVA with Bonferroni post-hoc p-value adjustments and pair-wise t-test comparisons. Further differences between subgroups were examined using independent sample t-tests, while paired samples t-tests were used for testing differences within each group. Categorical variables were tested for between-group differences using Chi-square or Fischer's exact tests and bivariate correlations were calculated with the Spearman's rho correlation coefficient. All S100B concentrations presented in the text are back-transformed values from the log₁₀ values used in the analyses. Descriptive data are presented as the mean with 95% confidence intervals (CI) of the distribution, while comparative data are presented as mean and the corresponding 95% CI of the mean. Based on the standard deviation from the baseline samples, the lowest true difference between the groups that could be identified was 0.017 ng/mL with at least 25 players in each group, with a power of 80% ($\beta=0.8$). All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS version 13.0, SPSS inc., Chicago, USA).

Results

Baseline Characteristics and Compliance

Baseline blood samples were drawn from 255 (88.2%) of the players who consented to participate prior to the 2004 season and 280 players (84.3%) prior to the 2005 season. Hence, 535 baseline samples were collected in total and the mean serum concentration of S100B was 0.045 (95% CI: 0.018 to 0.11) ng/mL. A total of 15 (2.8%) of the baseline samples were equal or above the cut-off at 0.12 ng/mL. Three repeated baseline measurements were performed on a total of 49 players and the mean baseline concentrations of S100B for the three different test days ranged from 0.049 (95% CI 0.026 to 0.093) ng/mL to 0.056 (0.028 to 0.11) ng/mL with a CV of 18.4%.

A total of 228 head impacts that met the inclusion criteria were identified on video from 352 matches. Sixty-nine (30.3%) of these were followed up with a blood sample within one hour after the impact (B1, N=65), or an additional blood sample the following day (B12, N=40), or both (N=37). The baseline characteristics and compliance with the sampling protocol for all four groups are presented in *Table 1*.

TABLE 1. Baseline characteristics and compliance with the sampling protocol for the players who experienced a head trauma in a football match (Head Impact), the players who participated in a football match without experiencing a head trauma (Match Control), the High-Intensive Exercise group which did not practise any heading and the Heading exercise group.

Variables	Head Impact (N=69)	Match Control (N=56)	High-Intensive Exercise (N=48)	Heading (N=46)
Age	28.1 (22.5 to 35.0)	26.2 (19.0 to 33.0)	26.1 (18.5 to 33.6)	26.1 (18.4 to 33.7)
Height in cm	185 (175 to 194)	183 (172 to 191)	182 (171 to 195)	183 (171 to 195)
Weight in kg	81.6 (70.8 to 93.0)	79.2 (70.0 to 90.0)	78.1 (63.5 to 94.1)	78.2 (63.0 to 94.4)
Nationality				
Norwegian or Scandinavian	55 (79.7%)	43 (87.8%)	39 (81.3%)	37 (80.4%)
Playing positions				
Goalkeeper	4 (5.8%)	3 (6.1%)	6 (12.5%)	6 (13.0%)
Central Defender	22 (31.9%)	13 (26.5%)	11 (22.9%)	11 (23.9%)
Full Wingback	11 (15.9%)	9 (18.4%)	5 (10.4%)	5 (10.9%)
Central Midfielder	11 (15.9%)	12 (24.5%)	13 (27.1%)	12 (26.1%)
Midfielder	4 (5.8%)	4 (8.2%)	6 (12.5%)	5 (10.9%)
Striker	16 (23.2%)	8 (16.3%)	7 (14.6%)	7 (15.2%)
Number of Headers				
Respective match/training	-	6.8 (0.0 to 16.0)	-	18.9 (7.0 to 33.0)
Compliance with the test protocol				
Baseline sample (BL)	60 (87%)	49 (88%)	48 (100%)	46 (100%)
One-hour sample (B1)	65 (94%)	49 (88%)	35 (73%)	36 (78%)
Twelve-hour sample (B12)	40 (58.0%)	46 (82.1%)	33 (69%)	28 (61%)
Post-training questionnaire	-	-	36 (75%)	35 (76%)
Minutes from impact/end of match/training to B1	77.7* (32.3 to 153.3) (N=29 [†])	33.2 (20.0 to 80.0) (N=48)	26.3 (12.6 to 45.8) (N=35)	23.0 (9.7 to 40.8) (N=36)
Hours from impact/end of match/training to B12	14.9 (10.5 to 24.8) (N=20 [†])	13.3 (11.4 to 14.7) (N=46)	20.6 (17.7 to 22.1) (N=33)	18.6 (16.7 to 22.5) (N=28)

*Significantly different, $p < 0.02$. [†]The exact sample time was not available for all the samples in the Head Impact Group.

As presented in *Table 2* only 13 (5.7%) of the 228 impacts were reported in as time-loss injuries to TISS, including 7 (3.1%) concussions (0.6 per 1000 playing hours). In the followed-up group, a total of 27 (39.1%) players reported having symptoms directly after the impact, but only 9 (33.3%) of these were taken out of play.

TABLE 2. Reported injuries and retrospectively classified concussions based on the Vienna concussion definition for the identified head impacts (N=228)

	Head Impacts. Post match follow up status groups	
	Not followed up	Head Impact S100B
N	159	69
Reported time loss injuries to TISS	3 (1.9%*)	10 (14.5%)
Concussion	2 (1.3%)	5 (7.2%)
Facial fracture	1 (0.6%)	2 (2.3%)
Other	0	3 (4.3%)
Loss of consciousness (LOC)	1 (0.6%)	4 (5.8%)
Post traumatic amnesia (PTA)	-	2 (2.9%)
Classified as concussions (Vienna definition) [†]	-	27 (39.1%)
Taken out of play due to concussion	-	9 (13.0%)

*Percentages are reported within each group, the followed up cases and the group not followed up.

[†]Retrospective classification based on symptoms reported by the medical personnel or the players themselves.

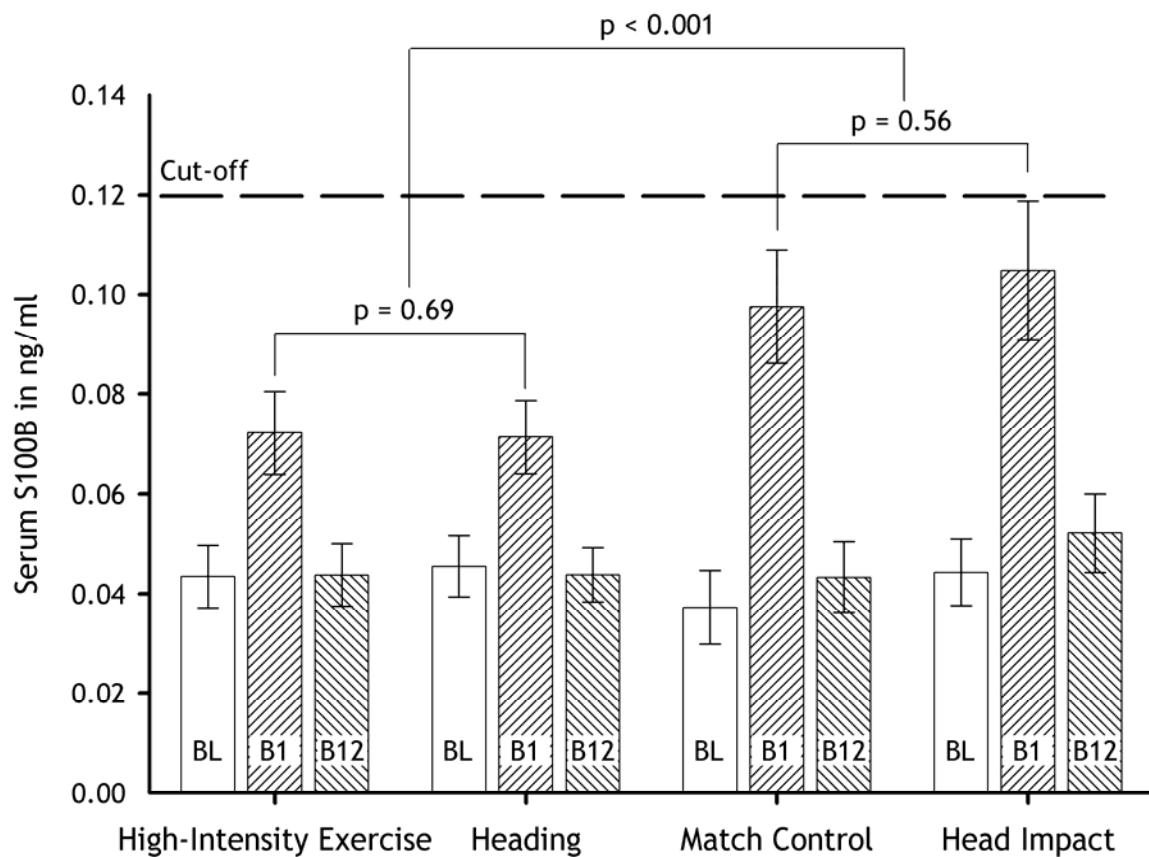
Two players experienced a head impact during the heading exercise session and were consequently excluded from further analyses. The serum concentration of S100B at baseline was not significantly different for any of the four groups (ANOVA, $p=0.408$).

Changes in the Serum Concentration of S100B

All groups had a significant increase in serum concentration of S100B between baseline and B1, and a similar significant decrease from B1 to B12 (*Figure 1*). Both match groups displayed higher B12 values compared to baseline, but only the B12 value for the Head Impact Group was significantly different from baseline (Baseline: 0.041 [95% CI 0.034 to 0.051] ng/mL, B12: 0.051 [95% CI: 0.43 to 0.59] ng/mL, $p=0.040$). For both training groups the B12 value had returned to their baseline level. However, it has to be emphasised that the

time from the end of the activity until B12 sampling the following morning was on average 5.8 (95% CI: 5.0 to 6.6) hours longer for the training groups compared to the match groups ($p < 0.001$), since the matches usually were played in the evenings while the training sessions took place around noon.

FIGURE 1. Mean S100B values in ng/mL for the Head Impact, Match Control, Heading and High-Intensity Exercise groups at baseline (BL), one hour (B1) and twelve hours post impact/match/training (B12). The error bars represent the 95% confidence interval of the mean.



Significant differences were neither seen between the two training groups nor between the two match groups for any of the sampling time points. The joint match groups (Match Control and Head Impact groups taken together) revealed a significantly higher mean serum S100B concentration at B1 compared to the joint training groups (*Figure 1*). A similar pattern was

evident for the Delta B1 values, where the joint match groups had a significantly higher increase from baseline compared to the joint training groups (Delta B1: Training Groups: 0.026 [95% CI: 0.020 to 0.031] ng/mL, Match Groups: 0.062 [95% CI: 0.052 to 0.073], $p < 0.001$). However, within the match and training groups, there were no significant differences in the Delta B1 values.

For the soccer players in the joint match group, a total of 39 (34.2%) B1 samples scored equal to or slightly above the cut-off (≥ 0.12 ng/mL), but they were equally distributed between the Head Impact and the Match Control groups (Chi-square: $p = 0.48$). Based on the symptoms reported either by the team medical personnel or by the players themselves, a total of 26 (37.7%) of the followed up impacts in the Head Impact Group were classified as concussions according to the criteria set by the 1st International Conference on Concussion in Sports in Vienna in 2001 (i.e. any impairment to neurological function after a head trauma) (7). Ten (38.5%) of these scored equal to or above the cut-off for B1 versus 14 (35.9%) of the 39 impacts that did not classify as concussions (Chi-square, $p = 0.83$). Only five B1 samples in the training group were equal or above the cut-off for B1. Although four out of these were within the High-Intensity group, the numbers were too small to test for any significant differences in the distribution.

The Effect of Heading and High-Intensive Exercise on Serum S100B

As shown in *Table 3*, the players in the Heading Group who reported the same number or more headers in the training session compared to a regular league match, had significantly higher Delta B1 values than the other players. However, this finding resulted from a significantly lower baseline serum level of S100B for the subgroup who reported the same or more frequent heading intensity. There was no significant difference in the serum concentration of S100B at B1 between the two subgroups. Within the High-Intensity Exercise

Group no differences were discovered with respect to the effect of the exercise intensity level compared to a regular match (*Table 3*).

TABLE 3. Serum concentration of S100B in ng/ml at all three test points for the High-Intensity Exercise Group and the Heading Group. Both groups are dichotomized according to self reported level of fatigue or number of headers compared to a regular match.

S100B sample	High-Intensity Exercise Group			Heading Group		
	Level of fatigue vs. match		p	No. of headings vs. match		p
	Less (N=19, 53%)	Same or more (N=17, 47%)		Less (N=10, 29%)	Same or more (N=25, 71%)	
Baseline	0.043 (0.035 to 0.053)	0.045 (0.036 to 0.056)	0.82	0.061 (0.043 to 0.087)	0.039 (0.034 to 0.045)	0.009
One hour sample (B1)	0.070 (0.060 to 0.081)	0.075 (0.061 to 0.092)	0.57	0.078 (0.057 to 0.11)	0.066 (0.060 to 0.072)	0.16
Twelve hour sample (B12)	0.041 (0.035 to 0.048)	0.047 (0.036 to 0.062)	0.40	0.052 (0.036 to 0.075)	0.041 (0.036 to 0.048)	0.20
Delta B1	0.025 (0.011 to 0.038)	0.032 (0.019 to 0.045)	0.73	0.016 (-0.005 to 0.036)	0.025 (0.020 to 0.031)	0.022

For the players in the Match Control Group there was a trend towards a positive correlation between the number of headers in the respective match and serum S100B at B1 (Spearman's $\rho = 0.28$, $p = 0.056$), but not for Delta B1 (Spearman's $\rho = -0.20$, $p = 0.89$). However, the players who headed ten times or more during the respective match (upper quartile: 0.045 [95% CI: 0.033 to 0.061] ng/mL) exhibited a trend towards a higher serum S100B concentration at baseline compared to the players who headed three times or less (lower quartile: 0.029 [95% CI: 0.019 to 0.044] ng/mL, $p=0.11$). When the number of all other head accelerating events and the number of headers were added, a significant correlation with serum S100B at B1 was found (Spearman's $\rho = 0.36$, $p = 0.012$), but still there was no correlation with Delta B1 (Spearman's $\rho = 0.025$, $p = 0.87$).

Discussion

This study followed elite **soccer** players for two seasons to determine whether minor head traumas in **soccer** cause detectable brain tissue injury. The serum concentration of S100B after head trauma was compared to the effect of heading, high-intensity exercise and playing a regular league match without any head trauma. Our main finding was that all conditions led to a moderate, but significant increase in serum S100B concentration, which returned to baseline levels within the next day. Although the increase was higher for the two match conditions compared to the two training conditions, there were no significant differences between the two match groups at any time point.

S100B and Minor Head Trauma

The post-match serum S100B levels after a head trauma were not different from levels measured after playing 90 minutes of professional soccer without experiencing any head impacts. The increase in both match groups was comparable to serum S100B levels measured in Swedish professional male and female soccer players after playing a regular match (54, 56). In addition, there was no difference between the Match Control Group and the Head Impact Group in the proportion of players with elevated serum S-100B levels. Even for the impacts that were classified as concussions based on their symptoms, the proportion of players with elevated levels was not different from the remaining Head Impact Group or the Match Control Group.

Data from the league injury surveillance system, which is administrated by Oslo Sports Trauma and Research Center, showed that thirteen of the head impacts recorded caused an injury (i.e. concussion or facial fracture) that kept the player away from regular matches and training for 1 up to more than 21 days. However, B1 samples were available for nine of these impacts and none of these samples were above the theoretical maximum serum level of

S100B which can be achieved by stress or exercise induced failure of the blood brain barrier only (32). In addition, the Head Impact Group's mean B1 level was below the values reported for patients admitted to hospital with minor head trauma (Glasgow Coma Scale 13-15) (9, 12, 27, 36, 41, 48, 53), and under the half of the mean serum S100B levels reported for minor head trauma patients with CT and/or MRI abnormalities (9, 12, 41).

However, there are some limitations which must be borne in mind when interpreting the results. Firstly, a possible source of bias is that only 69 (30%) out of the 228 head impacts were followed up. After numerous efforts towards the teams and their medical personnel, we identified that the main reason for the low compliance was that the players were reluctant to be tested after the match mainly because they regarded the impacts as trivial. Analyses of all the impacts identified from the match videos revealed that 24.6% of the players who were followed up with blood samples after a head impact, did not return to play compared to 8.8% of the cases where the impacts were not followed up. Thus, a player who was taken out of play was nearly three times more likely to be followed up compared to the ones who returned to play. Consequently, the followed-up group was likely to include a higher proportion of more severe impacts, and accordingly 39.1% of these were retrospectively classified as concussions. Nevertheless, the majority returned to play after the head impact, indicating that both the players and the team medical personnel regarded the majority of the impacts as benign.

Secondly, the samples of both the Head Impact and the Match Control groups were drawn within an hour after the end of the match, although the head impacts occurred on average 56 minutes prior to the end of the match. The biological half-life of S100B in serum has been reported to be as short as 25.3 (95% CI: 15.3 to 35.3) minutes (28), and consequently an increase in S100B caused by the head impact would decrease substantially during the time from the impact until the end of the match.

Nevertheless, even when considering these limitations, the head impacts did not have an additive effect on the S100B concentration when compared to playing a **soccer** match only, indicating that the head impact did not cause substantial nervous tissue injury.

S100B and Soccer Play

The present study showed an increase in serum S100B after playing a regular match irrespective of whether or not the players had experienced any head impacts. In addition, about 35% of these cases the values were above the suggested cut-off (0.12 ng/mL) used for severity screening of minor head trauma patients in hospitals (12). A somewhat smaller increase was found after a high-intensity exercise without heading. Comparable increases in serum S100B have been reported after various physical activities where head traumas and other sudden head-accelerating events like heading, are rare, such as long-distance running (42), swimming (16) and basketball (55). The effect of physical activity on the serum level of S100B and the source of S100B release into the serum under these circumstances are unresolved (4, 5, 17, 54, 56). Extracerebral sources of S100B are well known, such as in chondrocytes, melanocytes and fat cells (68), but the concentrations in these cells are very small compared to astroglial and Schwann cells (24, 27). Although, an increase of S100B has been reported in patients with multi-trauma (5) or isolated single bone fractures (66) without an obvious direct head injury, this does not exclude an indirect disturbance of nervous cells via inflammatory factors like cytokines released in high amounts in these trauma situations (20, 31, 37). Similar short term cell activating effects may occur during intensive physical work-out and could explain the increases reported after exercise, indicating that the source for S100B in serum may indeed be the nervous tissue (44, 54, 58).

Severe damage to the brain is typically accompanied by a breakdown of the blood-brain barrier function (32), but recent studies have established that the blood-brain barrier also can be disrupted under physical activity, such as prolonged moderate exercise in warm conditions

(67) and 30 minutes of forced swimming (animal study, (52)). Based on mathematical modelling of the S100B kinetics across the blood-brain barrier, Marchi et al. (32) proposed that up to a level 0.34 ng/mL, serum S100B is primarily a marker of increased blood-brain barrier permeability, whereas higher values are associated with neuronal damage and poor patient outcome. In comparison, the highest value in our study was 0.33 ng/mL, and this sample was drawn 20 minutes after a league match from a midfielder player in the Match Control Group. He did not head the ball during that particular match and his baseline and B12 samples were normal.

Exertion, stress and increased circulating levels of epinephrine have also been shown to increase the blood-brain barrier permeability (1, 25, 51) thus enabling a rise in the serum S100B levels. Playing a competitive match is associated with high levels of stress, adrenaline and physical intensity which it is difficult to mimic in a regular training session. This was reflected in the post-training questionnaire, where 53% of the players reported a lower level of fatigue after the training session compared to a league match. Hence, the higher B1 values for the match groups compared to training groups in our study could be due to different level of exertion only.

Properties of the S100B measurement procedure could also have affected the results. The measured S100B in this study refers to the summed concentrations of the S100B monomers in S100A1B and S100BB. A recent study has found a higher increase in S100A1B in patients with minor head traumas compared to patients with minor orthopaedic injuries, while the increase in S100B was equal for the two different groups (41). Nevertheless, although the specificity for brain injury after a minor head trauma seems to be higher for S100A1B, the sensitivity to detect brain tissue damage is comparable for S100B.

S100B and Heading

In our heading exercise session, the idea was to minimize the effect of physical activity and subsequently tease out the effect of heading only. However, after correcting for the difference in the S100B baseline values within the Heading Group, we could not detect any relationship between S100B and perceived heading intensity. Furthermore, we found no correlation between the observed number of headers and head accelerating events and the Delta B1 values as previously reported in the studies on the Swedish soccer players (54, 56). Yet, a closer examination of the baseline levels for the upper quartile compared to the lower quartile with respect to the number of headers in the match, revealed a trend towards higher baseline levels for those who headed most frequently and consequently this could cause a subsequent bias of the delta values for our Match Control Group. A plausible explanation could be that the baseline samples were collected during a training camp where the players had two or three training sessions per day, and although the baseline blood sampling was performed in the morning before training, there could be some effects left from the training sessions the day before for the most frequent headers.

The goalkeepers also represent a problem in these correlations. Goalkeepers practically never head the ball, and their level of exertion during a match is lower compared to the outfield players (6, 47). Consequently the goalkeepers will be grouped among the low frequency headers, and there is a chance that the correlation between number of headers and the increase in S100B would be confounded by differences in physical activity during the match. The studies by Stalnacke et al. (54, 56) provide no information regarding the goalkeepers and their results are therefore difficult to compare directly with ours.

In contrast to our results from the heading session, Mussack et al. (39) found that an exercise session with repetitive controlled headers led to a higher transient increase in serum S100B than an exercise session only. However, this study was performed on young amateur players

and a significant increase was only seen for the youngest group of players (from 12 to 15 years). According to Kirkendall and Garrett (29), coaches do not incorporate heading in the training sessions until the players are 12 years or older. Consequently controlled repetitive heading for 55 minutes was most likely a heavier exposure for the youngest players in the study of Mussack et al. (39) compared to the more experienced 16-17 year old players. This is in line with biomechanical studies of heading where brain accelerations during normal heading by adult players have been estimated to average less than 0.1% of the accepted levels required to produce brain injury in a single impact, while “accidental” heading and heading with poor technique could cause brain accelerations within the concussive range (8).

Conclusion

The serum level of S100B increases transiently after soccer training and soccer matches. There is a possible additive effect of heading and high-intensity exercise, but minor head impacts do not seem to cause an additional increase in S100B beyond the levels seen after a regular game. Thus, there is no evidence suggesting that there is significant brain tissue injury after these minor head impacts in soccer. However, for clinical use S100B is not an ideal marker for brain injury in athletes due to the confounding effect exercise alone.

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