ORIGINAL ARTICLE

Serum concentrations of two biochemical markers of brain tissue damage S-100B and neurone specific enolase are increased in elite female soccer players after a competitive game

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Background: It is a matter of debate whether or not ordinary heading of the ball in soccer causes injury to brain tissue.

Objective: To analyse concentrations of the biochemical markers of brain tissue damage S-100B and neurone specific enolase (NSE) in serum of female elite soccer players in association with a competitive game.

Methods: Venous blood samples were obtained from 44 female soccer players before and after a competitive game for analysis. The number of headers and trauma events (falls, collisions, etc) was assessed from videotape recordings for each player.

Results: Concentrations of both brain damage markers were increased after the game (S-100B, 0.18 (0.11) v 0.11 (0.05) µg/l ($p = 0.000$); NSE, 10.14 (1.74) v 9.05 (1.59) µg/l ($p = 0.001$)). There was a significant correlation between changes in S-100B concentrations and both the number of headers ($r = 0.430$, $p = 0.004$) and the number of other trauma events ($r = 0.517$, $p < 0.001$).

Conclusion: The concentrations of both S-100B and NSE were increased by game associated activities and events. The increases in S-100B concentration were significantly related to the number of headers and other trauma events, which indicates that both these factors may have contributed to these increases.

Soccer is one of the most popular sports in the world (200 million active players) played by both sexes, in all age groups and at all skill levels.¹ It was originally set up for men during the second half of the 19th century, but during the last 20–30 years, the game has increased in popularity among women, who now account for 22% of soccer players world wide.² Soccer has always been associated with a high injury rate,³ and the increase in participation of women has raised the issue of whether female soccer players, because of their lower body mass, may be at greater risk of injury than male players.⁴ During recent years, some studies of the injury pattern in both men and women have been published. They indicate that female players do in fact run an increased risk of injury in general during soccer play.⁵

Soccer is a contact sport and like other contact sports (boxing, rugby, ice hockey, etc) it is characterised by a comparatively high incidence of mild traumatic brain injuries (MTBIs)/concussions.⁶ In women's soccer, concussions account for 11% of all game injuries compared with 9% of game injuries in men's soccer.⁷

During recent years concern has been raised over whether or not ordinary soccer play and heading of the ball is dangerous to the brain. Some studies have reported cognitive dysfunction and neurological abnormalities in both retired and active soccer players.^{8–10} On the other hand, other studies have failed to show any signs of chronic brain damage in soccer players.^{11–12} All these studies report consequences of mild head injuries/concussions in male soccer players.^{8–10} In contrast, only a few studies investigating head trauma in female soccer players are available,^{13–14} and none of them deals with effects of concussions.

We aimed therefore to study female soccer players during a competitive game with respect to head trauma events (including heading) and simultaneously to assess serum

concentrations of two biochemical markers for brain damage, S-100B and neurone specific enolase (NSE), which have been found to be sensitive indicators of acute brain tissue injury.¹⁵ S-100B is a calcium binding protein which is present in high concentrations in the glial cells of the central nervous system,¹⁶ and NSE is a cytoplasmic enzyme occurring predominantly in neurones.¹⁵ Both these markers may also be of predictive and prognostic value with respect to outcomes after head trauma—for example, long lasting neurocognitive abnormalities¹⁷—and the occurrence of disability.¹⁸

METHODS

Subjects

The players of four female elite soccer teams in the Swedish Elite Soccer League (two competitive games altogether) were asked to participate in this study. Forty four players who gave their informed consent comprised the sample of this investigation. The characteristics of the players were (mean (SD) values): age, 23 (3.0) years; height, 168 (5) cm; weight, 62 (5.5) kg; body mass index, 22.2 (1.46) kg/m². Information on the number of previous concussions was obtained for 26 players (number of previous concussions, 1.4 (1.9) (range 0–5)).

Blood sampling and analysis of biochemical markers

Before and immediately after a competitive game, venous blood samples were taken from the players. The blood samples were allowed to clot, and, after centrifugation and transport, they were frozen and stored at -78°C until analysis. All samples were analysed at the Karolinska Hospital, Department of Clinical Chemistry, Stockholm, Sweden. Serum protein S-100B was analysed (as one batch)

Abbreviations: MTBI, mild traumatic brain injury; NSE, neurone specific enolase

using an immunoluminometric assay kit (Liaison Sangtec 100; Sangtec Medical, Bromma, Sweden), which measures A1B isoforms (present mainly in glial cells) and BB isoforms (occurring mostly in glial cells and Schwann cells) of the protein S-100. NSE was also measured by an immunoluminometric assay based on monoclonal antibodies (Liaison NSE; Sangtec Medical).

Assessment of heading and trauma events

Both competitive games were recorded on videotapes by professional photographers. Two independent investigators analysed the video recordings separately using criteria agreed on beforehand to assess the accelerations/decelerations to which the heads of the players were subjected. The number of acceleration/deceleration events—that is, headers with jumps/collisions/falls; headers without jumps, collisions, and falls; jumps, collisions, falls without head contact denoted other trauma events—was estimated for each player. Pearson product moment coefficients were calculated for headers ($r = 0.903$, $p = 0.000$) and other trauma events ($r = 0.825$, $p = 0.000$); they indicated acceptable inter-rater reliability. The mean values of different types of event were calculated for each player. The investigators were blind to the laboratory data on the players.

After the game, the players were asked to estimate the number of headers they made during the game using a scale 0–3 (0 = no heading at all, 1 = a few headers, 2 = intermediate number of headers, 3 = large number of headers).

Statistical analysis

All data were analysed with the statistical package for the social sciences (SPSS) version 12.0. The Wilcoxon signed

ranks test was used for the study of paired observations. Bivariate correlations were calculated with the Spearman rank coefficient. The level of statistical significance was $p < 0.05$. The study was approved by the ethics committee of Umeå University, Sweden.

RESULTS

Serum concentrations of S-100B and NSE

Figure 1 shows the results of the analysis of serum concentrations of S-100B and NSE before and after the game. For most of the players, S-100B and NSE concentrations were significantly increased after the game (S-100B, 0.18 (0.11) ν 0.11 (0.05) $\mu\text{g/l}$ ($p = 0.000$); NSE, 10.14 (1.74) ν 9.05 (1.59) $\mu\text{g/l}$ ($p = 0.001$)).

S-100B, NSE, headers and other trauma events

The mean number of headers with jumps/collisions/falls—that is, the mean value of the two assessments from videotape recordings by the two investigators—was 1.9 (2.2) (range 0–9.5), and the mean number of headers without jumps/collisions/falls was 1.2 (1.8) (range 0–9.5). The mean number of pooled headers was 3.1 (3.6) (range 0–15.5) per player (six players did not perform any headers at all). The mean number of other trauma events was 5.8 (2.5) (range 0–11) per player. The changes in S-100B and NSE were correlated with the number of different types of trauma event. The changes in S-100B correlated significantly with the number of headers (headers without jumps/collisions/falls, $r = 0.307$, $p = 0.042$; headers with jumps/collisions/falls, $r = 0.474$, $p = 0.001$; all headers pooled, $r = 0.430$, $p = 0.004$) and with the number of other trauma events ($r = 0.517$, $p = 0.001$). When all types of trauma event were pooled, $r = 0.515$ ($p < 0.001$). In contrast, no significant correlation was found for the changes in NSE (all headers, $r = 0.169$, $p = 0.27$; other trauma events, $r = 0.205$, $p = 0.18$; all types of trauma events, $r = 0.167$, $p = 0.27$). Figure 2 shows correlations between changes in serum concentrations of the markers and pooled headers and other trauma events.

Self estimated frequency of heading

All 44 players rated their heading frequencies. Three players (7%) reported no heading, 24 (56%) performed a few headers, 11 (25%) estimated their heading as intermediate, and the remaining six (14%) reported a large number of headers. The self estimated frequency of heading was found to correlate significantly with the mean number of pooled headers ($r = 0.526$, $p = 0.000$).

The player with the greatest increase in S-100B during the game (0.54 $\mu\text{g/l}$) estimated that she had performed a large number of headers, although the number of headers was only 1.0 and the number of other trauma events only 3.5, as assessed from the video recording. One of the players had an extraordinary head trauma (collision) when she sustained a mild concussion without loss of consciousness. She had the second highest increase in S-100B during the game (0.25 $\mu\text{g/l}$), and rated her heading frequency as intermediate.

DISCUSSION

This study shows that serum concentrations of both S-100B and NSE were increased in female elite soccer players as the result of activities connected with a competitive game. Furthermore, the changes in S-100B concentration after the game correlated significantly with both the number of headers and the number of other trauma events that had occurred during the game.

Serum concentrations of S-100B

The serum concentrations of S-100B after the game were in general lower than those found in patients after concussions/

Figure 1 Serum concentrations of the biochemical markers of brain damage S-100B (A) and neurone specific enolase (NSE) (B) before and after a competitive game of soccer. Symbols corresponding to each player are connected with a line.

Figure 2 Correlations between changes in serum concentrations of S-100B (A,B) or neurone specific enolase (NSE) (C,D) and trauma events (A,C) or headers (B,D).

MTBIs.^{18–19} The range of induced changes in S-100B in this study is in accordance with the changes observed in both male athletes—for example, elite soccer players,²⁰ ice hockey players, basketball players,²¹ and swimmers—and healthy male subjects after long distance running.^{22–23} This study shows that female athletes are similar to male athletes with respect to changes in serum S-100B concentration induced by sporting activity.

Serum concentration of NSE

The concentrations of NSE were significantly increased in the blood samples obtained after the games. These increases were generally lower than those observed in patients early after mild head injury/MTBI/concussion,^{17–18} but of the same magnitude as the concentrations in sera of male soccer players after a game.²⁰

Headers and other trauma events

The mean number of headers was 3.1, which is in the same range as in a parallel study of male soccer players (3.3).²⁰

The changes in S-100B concentrations correlated significantly with both the number of headers ($r = 0.430$, $p = 0.004$) and the number of other trauma events ($r = 0.517$, $p < 0.001$). These findings are also in accordance with the parallel study of male soccer players.²⁰

Causes and sources of the increases in serum S-100B and NSE concentrations

S-100B and NSE have been suggested to be promising biochemical markers of brain tissue damage.¹⁵ However, a number of studies have shown that they exhibit rather low specificity as markers of brain damage, as S-100B in serum

What is already known on this topic

- Increases in serum concentrations of S-100B are elicited by sporting activity such as running, ice hockey, basketball, and soccer in male athletes

What this study adds

- Female soccer players exhibit similar increases in serum S-100B concentration and similar relations between increases in S-100B concentration and the number of headers and other trauma events to those observed in male players in association with game play

may be raised in patients with multitrauma,²⁴ fractures,^{25–26} or during surgery.²⁷

Examples of extracerebral origins of S-100B are melanocytes and fat cells,²⁸ and NSE occurs in erythrocytes and extracerebral cells with neuroendocrine function.²⁹ Contributions to the serum increases observed in this study from extracerebral sources of S-100B and NSE cannot be disregarded.

However, it is possible that the source of the increase in serum S-100B concentration is nervous tissue (brain tissue). Even so, it is not necessarily the case that these increases reflect damage to brain tissue (glial cells), as S-100B may increase in serum solely as a result of opening/increased permeability of the blood-brain barrier,³⁰ which may occur during exercise (studies in animals³¹) and stress.³² Such a mechanism may come into play in all types of exercise and sports activities, and it may explain the increases in serum S-100B concentrations observed in studies on subjects without any obvious head trauma—for example, swimmers, runners, basketball players, ice hockey players.^{21–23}

However, in several studies on team athletes, significant correlations have been found between elements of the game associated activities and increases in serum S-100B. Thus game induced increases in serum S-100B concentrations have been shown to correlate significantly with the number of jumps for male basketball players,²¹ the number of headers for both male and female soccer players (Stålnacke *et al*²⁰ and the present study), and the number of other trauma events (falls, collisions, etc) for soccer players of both sexes (Stålnacke *et al*²⁰ and the present paper). Transient increases in serum S-100B have also been shown after a 55 minute session of controlled heading in soccer players.³³ Accordingly, there is a body of evidence that both direct head trauma (heading) and acceleration/deceleration of the body without head trauma (falls, collisions, jumps, etc) during ordinary play in soccer and basketball may contribute to release of S-100B into the blood. There is a need for further study of the causes, origins, mechanisms, and consequences of these increases.

Sex aspects

To our knowledge, this is the first investigation of S-100B and NSE markers in female elite soccer players. The study focused mainly on head contact (headers) and acceleration/deceleration of the players' bodies. The significance of generally smaller body size, greater ball to head ratio,¹³ and weaker neck muscles in female players compared with male players¹³ to the risk of injury in general and head injury in particular is still a matter of debate. Our study provides information showing that female elite soccer players resemble male players in most respects—that is, game induced increases in serum S-100B and NSE, presence of significant correlations between the number of headers or other trauma events and game induced increases in serum S-100B concentration, and the number of headers during the game.

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