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ORIGINAL ARTICLE

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Background: The possible injurious effect to the brain of heading in soccer is a matter of discussion.

Objective: To determine whether standardised headings in soccer are associated with increased levels of biochemical markers for neuronal injury in cerebrospinal fluid (CSF) and serum.

Methods: 23 male amateur soccer players took part in a heading training session involving heading a ball kicked from a distance of 30 m at least 10 m forward. Ten players performed 10 and 13 players performed 20 approved headings. The players underwent lumbar puncture and serum sampling 7–10 days after the headings. The study also included 10 healthy male non-athletic control subjects. CSF was analysed for neurofilament light protein, total tau, glial fibrillary acidic protein, S-100B and albumin concentrations. Serum was analysed for S-100B and albumin.

Results: None of the biomarker levels were abnormal and there were no significant differences between any of the three groups, except for a slightly increased CSF S-100B concentration in controls compared with headers. Biomarker levels did not correlate with the number of headings performed.

Conclusion: Repeated low-severity head impacts due to heading in soccer are not associated with any neurochemical signs of injury to the brain.

There has been growing controversy about the possible injurious effects to the brain of repeated low-severity head impacts, such as when heading a football.¹ However, there are few scientific data substantiating these concerns with direct evidence for neuronal damage in response to the impact. In a series of retrospective studies including retired Norwegian soccer players, cognitive deficits were noted.^{2,3} Similar results have been obtained in some American studies.⁴ In contrast, other studies have failed to show any signs of brain damage in soccer players.^{5,6} The conflicting data may be explained by methodological problems that may affect some of these studies, including the lack of pre-injury data, selection bias, failure to control for acute head injuries, lack of blinding of observers, and inadequate controls.

Two recent observational studies that determined serum concentrations of two biochemical markers for brain damage, S-100B and neurone specific enolase, in elite soccer players before and after a competitive game showed that both markers were increased by game-related activities and that the S-100B levels correlated with the number of headings.^{7,8} However, S-100B may also be released from extracerebral tissue in response to trauma not involving the brain,^{9,10} which makes the data somewhat uncertain.

The best-established biomarkers for neuronal injury are those analysed in cerebrospinal fluid (CSF), which is in direct contact with the brain interstitial fluid without any barriers. Hence, changes in neuronal function, metabolism and survival and host responses to neuronal injury are more likely to be directly reflected in the CSF proteome than in peripheral blood. CSF biomarkers of brain injury include total tau (T-tau) and neurofilament light (NF-L) proteins that reflect axonal injury,^{11,12} and the intermediate filament protein glial fibrillary acidic protein (GFAP) and the calcium-binding protein S-100B that reflect injury to glial cells.^{13,14} For example, in stroke there is a marked, transient increase in the CSF levels of these proteins that correlates with the infarct size.^{15–17} Also, very minor strokes can be detected.¹⁷ In amateur boxing, we recently

found evidence for subclinical injury to brain tissue using these markers.¹⁸ The increase was most pronounced for NF-L and correlated with the number of head punches received, although no boxer had been knocked out. Another possible challenge to the brain, electroconvulsive treatment, does not result in any change in CSF biomarker levels,^{19,20} and there is now a consensus that this procedure is harmless to the central nervous system.²¹ Hence, CSF T-tau, NF-L, GFAP and S-100B are sensitive and specific indicators of damage to the brain. Here, we examined the possible brain-damaging effects of heading in soccer by conducting an experimental study on CSF biomarker levels in amateur players who performed standardised headings as a part of a training session.

SUBJECTS AND METHODS

Subjects and experimental setting

Twenty-three male amateur soccer players participated in a training session that involved heading a ball kicked from a distance of 30 m in front of the player. The players were required to head the ball from a standing position at least 10 m forward with an allowed deviation of 45° to each side (fig 1). The 30 m distance translates well to a regular corner kick. Ten players performed 10 and 13 players performed 20 approved headings. The total number of headings (approved and unapproved) ranged from 11 to 57. The ball used was an Adidas Teamgeist 2006 FIFA World Cup Official Match soccer ball fulfilling the FIFA approved standard of a weight between 420 and 445 g and a circumference of 68.5–69.5 cm. The ball was inflated to a pressure of 900 g/cm² (FIFA approved standard 600–1100 g/cm²).

Seven to 10 days after the training session the participants underwent lumbar puncture (LP) through the L3/L4 interspace for collection of CSF, and venous puncture for collection of

Abbreviations: CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; LP, lumbar puncture; NF-L, neurofilament light protein; T-tau, total tau

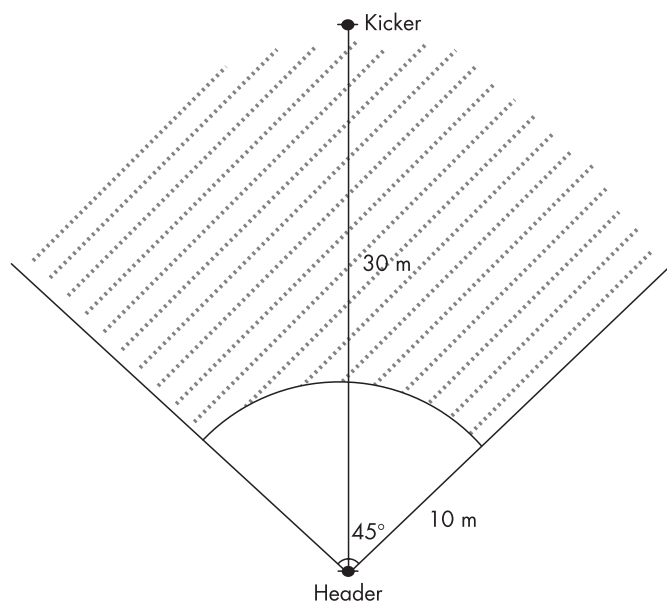


Figure 1 Schematic illustration of the experimental setting. One player kicks the ball at a distance of 30 m to the header, who heads the ball from a standing position at least 10 m forward. A deviation of 45° to each side is allowed. The header is allowed to run to a standing position within a 5 m square to position himself for the heading. The hatched lines demark the area in which the ball should land if a heading is to be approved.

serum. The 7–10-day time point was chosen as the optimal point at which to detect a change in biomarker levels as a result of the headings on the basis of CSF biomarker kinetics in a study on stroke.¹⁶ CSF was collected in polypropylene tubes, aliquoted and stored at -80°C pending analysis. The participants were examined physically and neurologically before the LP. All were healthy and none showed any signs of focal neurological injury. The study also included 10 healthy, male, age-matched, non-athletic control subjects who underwent LP and serum sampling but who did not participate in any activities involving head impacts. One control was excluded owing to marked blood contamination of the CSF. The study was approved by the ethics committee of Göteborg University, and written informed consent was obtained from all participants.

Biochemical analyses

CSF T-tau concentration was determined by a sandwich ELISA (Innotest hTAU-Ag, Innogenetics, Gent, Belgium) specifically

constructed to measure all tau isoforms irrespectively of phosphorylation status, as previously described.²² CSF concentrations of NF-L and GFAP were analysed using previously described ELISA methods.^{14–17} The detection limit of the NF-L ELISA was 125 ng/l. Serum and CSF levels of S-100B were determined by an electrochemoluminescence immunoassay using the Modular system and the S100 reagent kit (Roche Diagnostics). Albumin levels in CSF and serum were measured by immunonephelometry on a Beckman Image Immunochemistry system (Beckman Instruments, Fullerton, California, USA). The albumin ratio was calculated as CSF albumin (mg/l)/serum albumin (g/l). The coefficients of variation for all biochemical analyses were $<10\%$.

Statistics

The statistical calculations were performed using SYSTAT 11.0 (SYSTAT Software GmbH, Erkrath, Germany). The non-parametric Kruskal–Wallis test was used for comparisons between groups for quantitative variables. The Pearson correlation coefficient was used for analyses of correlation between biomarker levels and number of headings. A value of $p < 0.05$ was considered significant.

RESULTS

No significant differences were found between CSF or serum biomarker concentrations between soccer players who had performed 10 or 20 approved headings (table 1). Likewise, there were no significant differences between either of these two groups and the control group, except for a slightly increased CSF S-100B concentration in the control group compared with players with 10 and 20 approved headings ($p = 0.049$ and $p = 0.008$, respectively, table 1). There were no correlations between the number of approved headings or the total number of headings and any of the biomarker levels (table 2), and there were no intraindividual correlations between any of the biomarkers in any of the groups or in the whole material (data not shown).

DISCUSSION

The current study is to our knowledge the first determined attempt to determine whether there is a direct injurious effect of heading on brain tissue as reflected by CSF levels of T-tau, NF-L, GFAP and S-100B, which are sensitive and specific markers for neuronal and astroglial injury.²³ Further, we assessed brain-barrier function using the albumin ratio, and serum levels of S-100B, which may reflect injury to the brain. In contrast to our earlier finding for amateur boxing, where increased levels of T-tau, NF-L and GFAP that correlated with the number of received head punches were detected,¹⁸

Table 1 Demographic and biochemical variables in soccer players and controls*

| Variables | Soccer players with 10 approved headings (n = 10) | Soccer players with 20 approved headings (n = 13) | Controls (n = 9)† |
|-------------------------------------|---|---|---------------------|
| Age (years) | 26 (19–32) | 23 (20–28) | 24 (22–27) |
| Total number of headings | 14 (11–20) | 23 (20–57) | 0 |
| Albumin ratio | 4.1 (2.4–9.3) | 3.9 (2.0–8.7) | 4.1 (2.5–6.3) |
| NF-L (ng/l) | <125 | <125 | <125 |
| T-tau (ng/l) | 315 (170–400) | 250 (190–420) | 320 (120–540) |
| GFAP (ng/l) | 265 (180–510) | 260 (190–330) | 280 (190–460) |
| S-100B in CSF ($\mu\text{g/l}$) | 0.87 (0.71–1.2) | 0.82 (0.48–1.3) | 1.1 (0.77–1.2)‡ |
| S-100B in serum ($\mu\text{g/l}$) | 0.060 (0.030–0.12) | 0.040 (0.010–0.07) | 0.040 (0.030–0.060) |

*Data are presented as median (range).

†One of the 10 controls was excluded because of bleeding caused by the lumbar puncture.

‡ $p = 0.049$ for controls vs players with 10 approved headings and $p = 0.008$ for controls vs players with 20 approved headings.

Table 2 Correlations between total number of headings and biomarker levels in the whole group of soccer players (n = 23)*

| Variables | Sample correlation coefficient | 95% CI | p Value |
|-----------------|--------------------------------|----------------|---------|
| Albumin ratio | 0.37 | -0.047 to 0.68 | 0.080 |
| T-tau | -0.004 | -0.42 to 0.41 | 0.98 |
| GFAP | -0.19 | -0.56 to 0.24 | 0.38 |
| S-100B in CSF | -0.019 | -0.43 to 0.40 | 0.93 |
| S-100B in serum | -0.35 | -0.67 to 0.069 | 0.099 |

CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; T-tau, total tau.

*No correlation for neurofilament light protein (NF-L) is presented since the NF-L concentration was <125 ng/l in all samples.

the results of this study suggest that standardised headings in soccer do not result in neuronal or astroglial injury. Notably, the CSF levels of these biomarkers are more likely to reflect damage to brain tissue in a sensitive and specific manner than the corresponding serum levels, which may be influenced by biomarker release from extracerebral tissues.^{9, 10}

The reason for the slightly increased S-100B levels in CSF from controls compared with soccer players is unknown, but it should be noted that they are well below the levels found in independent control populations that were used when establishing clinical reference values for this analyte in our laboratory (CSF S-100B <1.7 µg/l).

It is estimated that professional soccer players head the ball five to six times a game.²⁴ Thus, the negative results in this study are unlikely to be because the number of headings performed by the participants was too low. Further, the distance of the ball flight of 30 m is similar to a regular corner kick, which is a common reason for heading in soccer. Finally, numerous studies from our laboratory and others show that the CSF biomarkers employed in this study are sensitive and specific markers for damage to the brain, including diffuse axonal injury and subclinical neuronal and astroglial injury in boxing.^{16-18, 23, 25-27}

In conclusion, this study suggests that the head-to-ball contact in a controlled heading does not cause any acute neuronal or astroglial injury. In agreement with this view, the majority of head injuries in soccer seem to be caused by

head-to-head contact with another player or goalpost collisions.²⁸ Thus, efforts to prevent brain injury associated with heading may be focused on reducing the likelihood of head-to-head or head-to-goalpost contact and improving the technique so that uncontrolled head-to-ball contact is avoided.

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Competing interests: None.

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What is already known on this topic

- CSF biomarkers can detect acute subclinical brain injury caused by amateur boxing.
- There are limited data available on the possible injurious effects to the brain of repeated headings in soccer.

What this study adds

- Standardised headings in soccer are not associated with any biochemical signs of neuronal or astroglial injury.
- The biomechanical effect on the brain of heading in soccer seems quite different from that caused by head punches in boxing.

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