Original Paper DOI: 10.5604/959284 Biol. Sport 2011;28:177-181

EFFECTS OF REPEATEDLY HEADING A SOCCER BALL ON SERUM LEVELS OF TWO NEUROTROPHIC FACTORS OF BRAIN TISSUE, BDNF AND NGF, IN PROFESSIONAL SOCCER PLAYERS

Accepted for publication 19.05.2011

AUTHORS: Bamaç B.[1], Tamer G.S.[2], Colak T.[1], Colak E.[3], Seyrek E.[3], Duman C.[4], Colak S.[3], Özbek A.[1]

[1] Kocaeli University, Faculty of Medicine, Department of Anatomy, Kocaeli, Turkey
[2] Kocaeli University, Faculty of Medicine, Department of Microbiology, Kocaeli, Turkey
[3] Kocaeli University, School of Physical Education and Sports, Kocaeli, Turkey
[4] Kocaeli University, Faculty of Medicine, Department of Biochemistry, Kocaeli, Turkey

Reprint request to:
Belgin Bamac,
Kocaeli University, School of Medicine, Department of Anatomy, Umuttepe, 41380, Kocaeli, Turkey.
Tel: 00 90 262 3037045
Fax: 00 90 262 3037510
E-mail: bbamac@hotmail.com

ABSTRACT: The present study determined the effects of heading training on serum nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) levels in soccer players. Seventeen professional level male soccer players (mean ± SD), age 24 ± 4.4 years, were recruited from a 3rd league team. Each player completed 15 approved headings in about 20-25 minutes. Venous blood samples were obtained from soccer players before and after the heading training for analysis. Levels of NGF and BDNF in the serum were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) kit. Mean ± SD serum NGF levels were 18.71 ± 3.36 $pg \cdot ml^{-1}$ before training and 31.41 ± 7.89 $pg \cdot ml^{-1}$ after training (p=0.000). Mean ± SD serum BDNF levels were 22.32 ± 3.62 $pg \cdot ml^{-1}$ before training and 55.41 ± 12.59 $pg \cdot ml^{-1}$ after training (p=0.000). In this study heading a soccer ball was found to cause an increase in serum concentrations of NGF and BDNF. We suggest that the microtrauma caused by repetitive heading and/or the course of survival of the injured neurons may lead to increased NGF and BDNF levels.

KEY WORDS: soccer players, neurotrophic factors, heading training

INTRODUCTION

Association Football (soccer) is the most popular and widespread sport in the world. A significant proportion of the injuries suffered in soccer are head injuries involving trauma to the brain [20]. Soccer players run the risk of incurring mild head injury from a variety of sources, including the intentional use of the head to play the ball, known as heading [24]. For over 100 years, heading was not considered seriously as an event which could result in head trauma, but now it has been suggested that the cumulative effect of head trauma caused by soccer heading may result in neurological damage and neuropsychological impairment [20,12]. A number of studies have shown the neurological, neuropsychological and cognitive deficits in amateur and professional soccer players due to repetitive heading. [27,28]. In a series of studies of active and retired soccer players, cognitive deficits were found to be associated with repeated heading [26,27]. On the other hand, some studies have reported no neurochemical evidence or neuropsychological impairment for brain injury caused by heading in soccer [19,25,28,29]. Hereby, from the available research, it is not fully known whether or not ordinary heading of the ball in soccer causes injury to brain tissue. Therefore, we decided to investigate whether heading the ball is associated with changes in serum levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which are known to be indicators of brain tissue damage.

Members of the neurotrophin family, including NGF and BDNF, can play dual roles: first, in neuronal survival and death, and secondly, in activity-dependent plasticity [8]. They act through their cognate tropomyosin-related kinase (trk) receptors (NGF/trkA, BDNF/trkB). While NGF/trkA interactions are believed to be important for cell survival during development and following injury [13], BDNF/trkB interactions appear to be neuroprotective following various forms of brain injury [10].

The BDNF and NGF released into the circulation are argued to be reliable markers displaying brain damage [10]. It has been suggested that these neurotrophins may provide neuroprotection by playing a role in the maintenance and survival of neurons after traumatic brain injury [9,14]. Hicks et al. [10] NGF demonstrated that a mild traumatic brain injury induced significant increases in hybridization levels for BDNF and trkB mRNAs in the hippocampus of rat brains. In football, contact to the head when heading the ball has the potential to cause traumatic brain injury [4]. Accordingly, the aim of this study was to investigate the relationship between expression of these two neurotrophins and repeated head impacts in professional soccer players.

MATERIALS AND METHODS

Participants. Seventeen male professional soccer players from a third division team volunteered to participate in this study. The study was approved by the local research Ethics Committee of our faculty and all players gave written informed consent before participating. They all trained three times per week and on average played one match per week. Table I displays physical characteristics of the players.

TABLE 1. PLAYERS' CHARACTERISTICS (N=17)

Age (years)	24.58±4.41
Height (cm)	174.88±6.18
Weight (kg)	73.00±9.93
BMI ($kg \cdot m^{-2}$)	23.78±2.12
Playing Experience (years)	12.29±4.89

Note: BMI - Body Mass Index

The players were asked to complete a questionnaire regarding age, height, weight, player position, playing experience in the league and history of previous concussion. Zero concussions were reported by the players. Participants were excluded from this study if they had a history of recent head trauma from soccer or other causes. No control group was studied; each participant served as his own control.

Field study

This study was conducted using an experimental set-up. First, a corner kick was taken (swinging corner from right side into the centre of the goal) at a distance of 30-35 m to the header (Fig. 1). When the kicker powerfully kicked the ball onto the field of play the header was required to head the ball from a jumping position. The headers contacted the oncoming ball at the frontal bone. This was representative of ordinary heading in soccer. Each player completed 15 approved headings in about 10-15 minutes. We chose to have players head 15 balls in an effort to imitate the maximum number of balls a player would head during a typical training session. The field test was performed on the same day for all players.

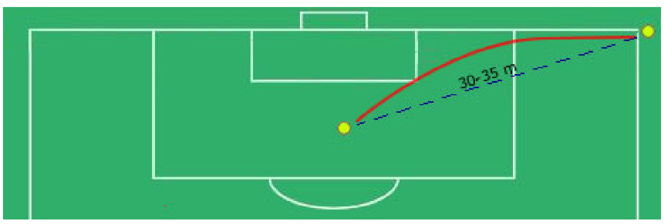

FIG. 1. SCHEMATIC ILLUSTRATION OF THE FIELD STUDY.

We used Adidas Teamgeist, having a mass of 430 g and specified ball pressure of 900 $g \cdot cm^{-2}$ (FIFA approved standard 600-1100 $g \cdot cm^{-2}$). Ball pressure was regularly checked during testing.

Biochemical analysis

In the present study, we assayed for peripheral serum NGF and BDNF levels via a blood (5 ml) withdrawal from the antecubital vein just before (resting) and after the heading training. Blood samples were collected aseptically and the serum was prepared and stored at −80°C for up to one month until BDNF and NGF analysis. Levels of BDNF and NGF in the serum were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Chemicon, USA) and results were expressed as $pg \cdot ml^{-1}$. All samples were assayed in duplicate, and the detection limit of the BDNF assay was 7.8 $pg \cdot ml^{-1}$ and NGF assay was 10 $pg \cdot ml^{-1}$ retrospectively.

Statistical Analysis

After tests for normality, the non-parametric Mann-Whitney U test was used to test differences between pre-exercise and post-exercise data. Means (SD) are given as descriptive statistics. SPSS (version 15.0) was used for statistical analyses (SPSS Inc., Chicago, Illinois, USA). A p value <0.05 was considered statistically significant.

Receiver operating characteristics (ROC) curve analysis was done for determination of specificity and sensitivity of the cut off values of all players (MedCalc, 9.4.2; Statistical Software for Biomedical Research). The area under the curve (AUC) was calculated with its standard error and 95% confidence interval.

RESULTS

Results of the biochemical measurements are displayed in Table 2. NGF and BDNF levels were significantly elevated in response to heading exercise.

TABLE 2. BIOCHEMICAL MEASUREMENTS OF SOCCER PLAYERS

	Pre-exercise	Post-exercise	p
NGF $pg \cdot ml^{-1}$	18.71 ± 3.36	31.41 ± 7.89	0.000
BDNF $pg \cdot ml^{-1}$	22.32 ± 3.62	55.41 ± 12.59	0.000

For the soccer ROC curve analysis, the area under the curve for BDNF was 1.000 (95% confidence interval [CI] 0.896 to 1.000; Fig. 2). Exploratory analysis of different cut points was performed with the ROC curve. The BDNF value with the most acceptable sensitivity and specificity capable of being raised was 27.6 $pg \cdot ml^{-1}$ (cut off point) (sensitivity 100%, specificity 100%) (Fig. 2). This value, with satisfactory sensitivity and high specificity, was regarded as a more suitable cut off point.

For the soccer ROC curve analysis, the area under the curve for NGF was 0.934 (95% confidence interval [CI] 0.793 to 0.989; Fig 3). Exploratory analysis of different cut points was performed

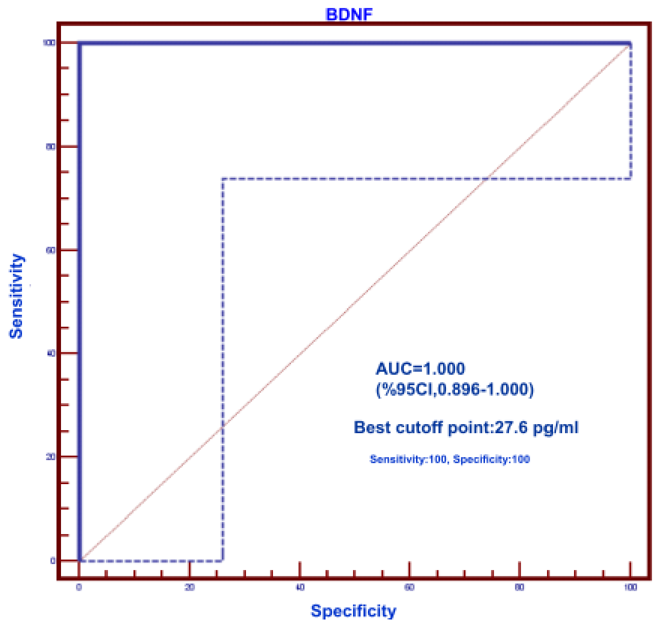

FIG. 2. RECEIVER OPERATING CHARACTERISTIC (ROC) ANALYSIS FOR THE ASSAY FOR BDNF
Note: AUC – area under the curve; CI – confidence interval).

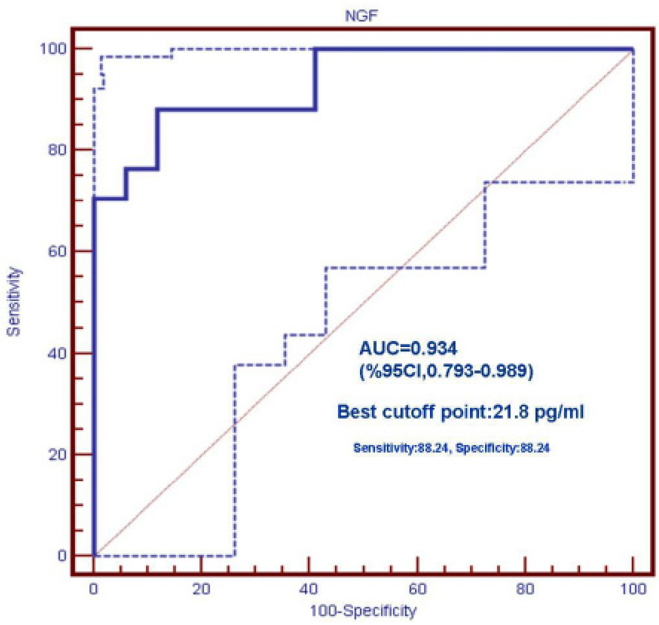

FIG. 3. RECEIVER OPERATING CHARACTERISTIC (ROC) ANALYSIS FOR THE ASSAY FOR NGF
Note: AUC – area under the curve; CI – confidence interval).

with the ROC curve. The NGF value with the most acceptable sensitivity and specificity capable of being raised was 21.8 pg·ml^{-1} (cut off point) (sensitivity 88.24%, specificity 88.24%) (Fig. 3). This value, with satisfactory sensitivity and high specificity, was regarded as a more suitable cut off point.

DISCUSSION

In recent years there have been several concerns regarding the potential consequences of effects of repetitively heading soccer balls on brain tissue. We aimed to study male soccer players to assess the level of circulating NGF and BDNF which have been found to be sensitive indicators of brain tissue repair. Concentrations of both neurotrophic factors in soccer players were increased after the training.

There might be a direct association between neural degeneration or recovery in the central nervous system and peripheral production of NGF and BDNF. Despite their size, these proteins can cross the blood–brain barrier (BBB), at least under experimental conditions [6,18]. BDNF is known to cross the blood-brain barrier in both directions [5] from the brain to the periphery and from the periphery to the brain, via a high-capacity saturable transporter system [17]. A positive correlation between BDNF levels in the brain and serum was described; therefore the blood levels of BDNF may reflect the brain levels and vice versa [30]. There is evidence from clinical trials that NGF like BDNF is transported over the blood-brain barrier and stored in platelets as well [1,18]. In the present study, we did not directly assess brain levels of NGF and BDNF but it seems that NGF and BDNF in the brain increased because of transport from the periphery, or both.

Exercise-related increases in circulating NGF and BDNF levels have been demonstrated in healthy humans [5,6]. Since exercise has been shown to cause increased levels of NGF and BDNF, one can question whether physical exercise during game-related activities in soccer may have caused the opening of the blood-brain barrier which might explain the release of NGF and BDNF into the blood. For this reason, we minimized the effect of physical activity to discriminate the effect of heading only. The soccer players in this study performed headers without doing any heavy warm-up activities.

Heading may be conceptualized as repeated subconcussive blows to the head due to speed and impact forces [28]. Professional soccer players play the ball with the head five to six times per game, on average [23], and of course many headers are performed in training [11]. Consequently, over a career of 20 years, a substantial cumulative burden is possible [20]. Ball speed can alter the severity of the impact to the head [22]. The average speed of kicking a soccer ball on the ground is 65-70 mph. Schneider and Zernicke [21] found that head injury risk was present with linear head acceleration caused by frontal and lateral heading impacts at medium velocities (frontal >70 km·h^{-1}, lateral >47 km·h^{-1}). At these speeds, the linear and angular accelerations would be higher, with greater potential for injury [16].

Increased levels of NGF should be interpreted with caution since their effect seems to depend on the respective receptors and could be neuroprotective or detrimental in nature [6]. In animal models of experimental brain injury, exogenous administration of NGF and other neurotrophic factors has been shown to prevent or significantly reduce severe neurological deficits, apoptosis, and brain-cell death [2]. Griescbach, G.S., [7] et al. have observed increases in BDNF levels after a mild Traumatic Brain Injury TBI in the developing rat, which may have strong implications for the mechanisms of post traumatic recovery. Chiaretti et al. [3] observed a significant and early up-regulation of NGF and BDNF in children with severe traumatic brain injury. They suggested that the expression of BDNF may represent an important early marker, whilst the NGF post-traumatic expression in the cerebrospinal fluid was predictive of a good clinical outcome. According to the same authors these neurotrophic factors act on different target cells and serve different functions in post-traumatic injury. There has been increasing concern that repeated impacts by heading could lead to significant head injuries in soccer players comparable to those of patients with accidental minor traumatic brain injury [15]. This mechanism may be postulated to explain the high NGF and BDNF level in our study. We speculate that the microtrauma caused by repetitive heading and/or the course of survival of the injured neurons may lead to increased NGF and BDNF levels.

CONCLUSIONS

Heading training has been shown to increase NGF and BDNF production in professional soccer players. However, these measurements in peripheral blood can reflect only a limited view of whole NGF and BDNF metabolism. Therefore additional studies are required in this area.

This study has certain limitations:
First, in the present study, contributions to the serum increases from extracerebral sources of NGF and BDNF should be kept in mind because neurotrophic factors are found not only in the brain, but in a range of tissue and cell types. It is not known whether the level of NGF and BDNF increases observed in this study is due to injury of brain tissue or whether it is only due to the opening or increased permeability of the blood-brain barrier.

Secondly, this study did not show whether the increase in neurotrophic factors was transient, and did not assess the time to return to baseline. Further study is required to investigate whether this increase occurs after ordinary heading training.

REFERENCES

1. Blandini F., Rinaldi L., Tassorelli C., Sances G., Motta M., Samuele A., Fancellu R., Nappi G., Leon A. Peripheral levels of BDNF and NGF in primary headaches. Cephalalgia 2006;26:136-142.
2. Chiaretti A., Antonelli A., Riccardi R., Genovese O., Pezzotti P., Di Rocco C., Tortorolo L., Piedimonte G. Nerve growth factor expression correlates with severity and outcome of traumatic brain injury in children. Eur. J. Paediatr. Neurol. 2008;12:195-204.
3. Chiaretti A., Piastra M., Polidori G., Di Rocco C., Caresta E., Antonelli A., Amendola T., Aloe L. Correlation between neurotrophic factor expression and outcome of children with severe traumatic brain injury. Intensive Care Med. 2003;29:1329-1338.
4. Dvorak J., Junge A., McCrory P. Head injuries. Br. J. Sports Med. 2005;39(Suppl. 1):i1-i2.
5. Ferris L.T., Williams J.S., Shen C.L. The effect of acute exercise on serum brain-derived neurotrophic factor levels and cognitive function. Med. Sci. Sports Exerc. 2007;39:728-734.
6. Gold S.M., Schulz K.H., Hartmann S., Mladek M., Lang U.E., Hellweg R., Reer R., Braumann K.M., Heesen C. Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. J. Neuroimmunol. 2003;138:99-105.
7. Griesbach G.S., Hovda D.A., Molteni R., Gomez-Pinilla F. Alterations in BDNF and synapsin I within the occipital cortex and hippocampus after mild traumatic brain injury in the developing rat: reflections of injury-induced neuroplasticity. J. Neurotrauma 2002;19:803-814.
8. Hennigan A., O'Callaghan R.M., Kelly A.M. Neurotrophins and their receptors: roles in plasticity, neurodegeneration and neuroprotection. Biochem. Soc. Trans. 2007;35:Part 2.
9. Hicks R.R, Numan S., Dhillon H.S., Prasad M.R., Seroogy K.B. Alterations in BDNF and NT-3 mRNAs in rat hippocampus after experimental brain trauma. Mol. Brain Res. 1997;48:401-406.
10. Hicks R.R., Martin V.B., Zhang L., Seroogy K.B. Mild experimental brain injury differentially alters the expression of neurotrophin and neurotrophin receptor mRNAs in the hippocampus. Exp. Neurol. 1999;160:469-478.
11. Jordan S., Green E., Galantly H., Mandelbaum B., Jabour B. Acute and chronic brain injury in United States National Team Football Players. Am. J. Sports Med. 1996;24:205-210.
12. Matser J.T., Kessels A.G., Jordan B.D., Lezak M.D., Troost J. Chronic traumatic brain injury in professional soccer players. Neurology 1998;3:791-796.
13. Mattson M.P., Scheff S.W. Endogenous neuroprotection factors and traumatic brain injury: Mechanisms of action and implications for therapy. J. Neurotrauma 1994;11:3-33.
14. Mochetti I., Wrathall J.R. Neurotrophic factors and central nervous system trauma. J. Neurotrauma 1995;12:853-870.
15. Mussack T., Dvorak J., Graf-Baumann T., Jochum M. Serum S-100B protein levels in young amateur soccer players after controlled heading and normal exercise. Eur. J. Med. Res. 2003;8:457-464.
16. Naunheim R.S., Standeven J., Bayly P. Cumulative effects of soccer heading are not fully known. BMJ 2003;15;327(7424):1168.
17. Pan W., Banks W.A., Fasold M.B., Bluth J., Kastin A.J. Transport of brain-derived neurotrophic factor across the blood-brain barrier. Neuropharmacology 1998;37:1553-1561.
18. Poduslo J.F., Curran G.L. Permeability at the blood brain and blood– nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF. Mol. Brain Res. 1996;36:280-286.
19. Putukian M., Echemendia R.J., Mackin S. The acute neuropsychological effects of heading in soccer: A pilot study. Clin. J. Sport Med. 2000;10:104-109.
20. Rutherford A., Stephens R., Potter D. The neuropsychology of heading and head trauma in Association Football (soccer): a rewiew. Neuropsychol. Rev. 2003;13:153-179.
21. Schneider K., Zernicke R. Computer-simulation of head impact-estimation of head-injury risk during football heading. Int. J. Sport Biomech. 1988;4:358-371.
22. Shewchenko N., Withnall C., Keown M., Gittens R., Dvorak J. Heading in football. Part 1: Development of response biomechanical methods to investigate head. Br. J. Sports Med. 2005;39 (Suppl. 1):i10-i25.
23. Sortland O., Tysvaer A.T. Brain damage in former association football players: An evolution by computed tomography. Neuroradiology 1989;31:44-48.
24. Stephens R., Rutherford A., Potter D., Fernie G. Neuropsychological impairment as a consequence of football (soccer) play and football heading: a preliminary analysis and report on school students (13-16 years). Child Neuropsychol. 2005;11:513-526.
25. Straume-Naesheim T.M., Andersen T.E., Dvorak J., Bahr R. Effects of heading exposure and previous concussions on

neuropsychological performance among Norwegian elite footballers. Br. J. Sports Med. 2005;39(Suppl. 1):i70-i77.
26. Tysvaer A. Head and neck injuries in soccer, impact of minor trauma. Sports Med. 1992;14:200-213.
27. Tysvaer A.T., Lochen E.A. Soccer injuries to the brain. A neuropsychologic study of former soccer players. Am. J. Sports Med. 1991;19:56-60.
28. Witol A.D., Webbe F.M. Soccer heading frequency predicts neuropsychological deficits. Arch. Clin. Neuropsychol. 2003;18:397-417.
29. Zetterberg H., Jonsson M., Rasulzada A., Popa C., Styrud E., Hietala M.A., Rosengren L., Wallin A., Blennow K. No neurochemical evidence for brain injury caused by heading in soccer. Br. J. Sports Med. 2007;41:574-577.
30. Zoladz J.A., Pilc A., Majerczak J., Grandys M., Zapart-Bukowska J., Duda K. Endurance training increases plasma brain-derived neurotrophic factor concentration in young healthy men. J. Physiol. Pharmacol. 2008;59 (Suppl. 7):119-132.