

## Effects of Acute Soccer Game on Serum Levels of Neurotrophins and Neurocognitive Functions in Male Adolescents

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The purpose of the present research is to investigate the effects of acute soccer game on serum levels of neurotrophins and neurocognitive function. The subjects of the research were 15 healthy male adolescents. The subjects underwent two experiments: one experiment in the soccer game treatment (SOC) condition, and the other in the self-study treatment (CON) condition. Blood samples were collected at three times: before treatment (Pre), after treatment (Post), and 2 hours post treatment (Post-2 h) for the analyses of serum brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and insulin-like growth factor-1 (IGF-1). For the measurements of neurocognitive functions, the Stroop Color-Word test was performed at blood collecting times. The results of the research showed that the serum levels of BDNF, NGF, and IGF-1 were significantly increased after the soccer game ( $p < 0.05$ ), and significantly higher in SOC than CON at Post ( $p < 0.05$ ). In the Stroop Color-Word test, significantly increased scores were observed in SOC at Post ( $p < 0.05$ ), and significantly higher in SOC than in CON at Post and Post-2 h ( $p < 0.05$ ). These results suggest that acute soccer game has positive effects on neurocognitive functions by increasing the neurotrophins.

**Key words** : Soccer, adolescents, neurotrophins, neurocognitive function

### Introduction

Recently, the effects of exercise on central nervous system (CNS) have been extensively investigated, exercise has been proved to enhance cognitive functions such as learning and memory from many preceding researches [23,24]. For example, exercise could induce positive change of brain structure [26], increase of glial cell generation [25], increase of angiogenesis [1], and increase of neurogenesis [37]. Especially, it has recently been considered that the increase of neurotrophins, which promote neurogenesis, plays an important role in the mechanism of the maintenance and improvement of brain cognitive function by exercise.

Neurotrophins, an important group of neurotrophic factors, include brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), and neurotrophin-6 (NT-6) [15]. They are involved in the proliferation, migration, survival, and differentiation of neurons, as well as in the regulation of synaptic plasticity [16,27]. It has been reported that the expression of BDNF and NGF with such roles and functions

were increased simply by regular aerobic exercise training and acute exercise. In detail, Cotman *et al.* (2007) reported that the level of BDNF mRNA in the hippocampus increased after running exercise [11]. In addition, according to Wu *et al.* (2008), treadmill running improved the proliferation and survival of neurons by increasing BDNF and tropomyosin-related kinase B (TrkB) [40]. Further, it was reported by Chae and Kim (2009) that 5 treadmill running exercises a week for 8 weeks increased NGF in the hippocampus in an experiment with Sprague-Dawley (SD) rats [9]; and by Oh *et al.* (2011) that an analysis of neurotrophin protein expression and receptor activity according to the quantity of acute exercise after spinal cord hemisection showed high levels of both after exercise—in all the cases of BDNF, NGF and NT-3 [27]. However, most of preceding researches, including those mentioned above [9,11,27,40], involved only animal models such as the rat. There were a few domestic and foreign researches involving human that monitored the change of circulating serum BDNF concentration to verify the effect of exercise training [16,31] and acute exercise [12,13]. In the case of NGF, there has been relatively insufficient research on the effect of exercise in human. Even in the case of BDNF, there has been very limited research, both domestic and foreign, for verifying the effect of a specif-

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ic type of exercise, such as soccer, in human.

Soccer is one of the team games that allows the cultivation of good physical strength, as well as the induction of desirable personality cultivation, while colleagues are playing together [20,22]. According to a research conducted by Park and Chae (2010), the physical education activity of soccer not only could improve the physical strength of male adolescents during their growth period, but also showed positive effect on mental health, such as compulsion, anxiety, sensitivity and depression [29]. Based on such results of preceding researches, it is considered that the physical education activity of soccer may be effective for the improvement of the brain health of adolescents in their growth period. Accordingly, present research aims first to examine the effect of the physical education activity of soccer—which is the most favored sports among the male adolescents in Korean middle and high schools, and in which Koreans showed high participation rate [10,19]—on serum BDNF and NGF. Another purpose of present research is to analyze IGF-1, which has been known to play an important role in the expression of BDNF and NGF [8,35], to verify the effect of exercise on serum neurotrophins in adolescents. In addition, the assessment of neurocognitive function by Stroop Color-Word test, which has been reported to be a useful index for verifying brain function [14,36], is expected to allow more clear verification of the effect of exercise-induced change of neurotrophins on brain function in human.

## Materials and Methods

### Subjects

Research subjects were male adolescents enrolled in D high school in S city who spontaneously expressed their intention to participate in the experiment, with sufficient understanding about its significance. A questionnaire survey was conducted to finally select 15 male adolescents, who were all in 2<sup>nd</sup> grade of the high school and were medically healthy, without a particular disease and with normal body composition. The finally selected subjects underwent 2 experiments—one experiment in the soccer game treatment condition (SOC), and the other in self-study treatment condition (CON). Table 1 presents their physical characteristics.

### Preliminary tests

Preliminary tests include measurements of height and body composition (body weight, fat mass, lean body mass,

Table 1. Physique characteristics of subjects

Variables	Subjects
N	15
Age (yr)	17.00±0.00
Height (cm)	175.47±4.88
Weight (kg)	64.72±5.65
Lean body mass (kg)	56.71±4.63
Fat mass (kg)	8.01±2.12
Body fat (%)	12.30±2.55

Values are means±SD.

and body fat). Height was measured with HD (STDK, Japan), and body composition was measured by bio-impedance body composition analysis with Inbody220 (Biospace, Korea). For accurate analyses, all subjects were asked to urinate prior to the tests and wear shorts during the test procedures. Foods and beverages were restricted to all subjects 2 hours prior to the test.

### General experimental design

As shown in Fig. 1, the experimental procedure involved Stroop Color-Word test and blood sampling before treatment (Pre), followed by participation in the soccer game treatment condition (SOC) and self-study treatment condition (CON), and then by the second Stroop Color-Word test and blood sampling after treatment (Post), which were carried out in the same way as Pre. After that, in both cases of SOC and CON, the subjects were allowed to take 2 hours of rest prior to the third Stroop Color-Word test and blood sampling at 2 h post-treatment (Post-2 h). In detail, the SOC treatment involved a warm-up exercise (stretching for 5 minutes), followed by a soccer game (the first and second halves, each for 45 minutes), and then by a cool-down exercise (stretching for 5 minutes). Whereas the CON treatment involved 2 sessions of self-study, each for 50 minutes. The subjects were allowed to take 10 minutes of rest between the first and second halves of the game during SOC treatment, and between the 2 sessions of self-study during CON treatment. In the meantime, in order to minimize the error of Stroop Color-Word test caused by the successive treatments, a cross-over design was adopted by having 7 out of the total of 15 research subjects receive SOC treatment first, while the remaining 8, CON treatment first. Also each treatment was separated by 7 days to avoid any transient effects on physiological and psychological conditions of the subjects.

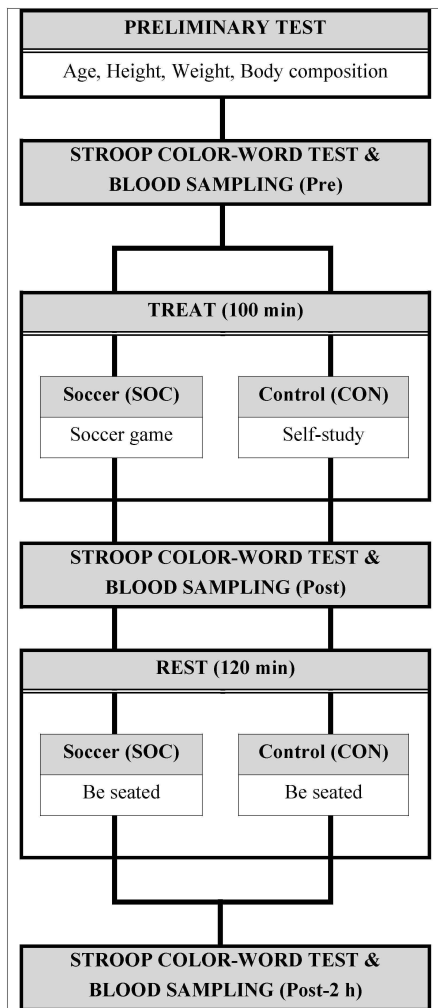


Fig. 1. Experimental design.

Neurocognitive function test

For the measurements of neurocognitive functions, the Stroop Color-Word tests were performed 3 times: Pre, Post, and Post-2 h as previously described [14,34]. The Stroop Color-Word test consists of 3 sections, all of which are variations of the same theme; subjects are required to read out loud, as quickly and accurately as possible, from a list of items for 45 s. Test 1, the Word test, requires that subjects read from a page containing three printed words: red, green, or blue. The words are printed in black ink and are listed in random fashion in five columns of 20 words each. No word is allowed to follow itself in a column. Test 2, the Color test, similarly consists of 100 items that are all written as XXXX and printed either in red, green, or blue ink. The subject states the color of the ink for each of the series of four X's. Test 3, the Color-Word test, consists of the same 100 words (red, green, or blue) as the first test, but printed in

colored ink, and the color of the ink and the printed word are never the same (for example, the word "blue" could be printed in red or green ink, but never blue ink).

Blood sampling and analyses

In the present research, we assayed for peripheral serum BDNF, NGF, and IGF-1 levels via a blood (8 ml) withdrawal from the antecubital vein before treatment (Pre), after treatment (Post), and 2 h post-treatment (Post-2 h). Blood samples were collected aseptically and the serum was prepared and stored at -80°C until the analyses. Levels of neurotrophins (BDNF, NGF) in the serum were determined by the commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (R&D Systems, USA) and results were expressed as pg/ml. Levels of IGF-1 in the serum were determined by the chemiluminescent immunoassay (CLIA) method, using Immulite 2000 IGF-1 kit (Siemens, USA). The measurement was performed by using the manufacturer's instructions and results were expressed as ng/ml.

Statistical analyses

All data were analyzed by SPSS/PC+ Ver. 18.0K statistical package. The values were expressed as mean ± standard deviation (SD). For statistical significance in levels of BDNF, NGF, IGF-1, and Stroop Color-Word tests (Word test, Color test, Color-Word test) for treatments and measuring periods were determined using a two-way repeated analysis of variance (ANOVA) for comparisons. One-way ANOVA was conducted to investigate the significant difference in variants according to each period in each treatment. Tukey's post hoc test was conservatively locate the significant differences. Also, paired t-test was conducted to investigate the significant difference in variants according to each treatment in measuring periods. The statistical significance was set at  $p < 0.05$ .

Results and Discussion

Changes in serum neurotrophins levels

The changes of neurotrophins (BDNF, NGF) according to the soccer game were presented in Table 2. The result of two-way repeated ANOVA of BDNF showed statistically significant difference in the period effect ( $p < 0.01$ ) and the treatment-by-period interaction effect ( $p < 0.05$ ). According to the post-hoc test, in the case of SOC condition there was

a significant increase of BDNF after treatment (Post) compared to before treatment (Pre), followed by a decrease eventually to the pre-treatment level at 2 h post-treatment (Post-2 h). And BDNF concentration was significantly higher in the case of SOC condition compared to CON condition at Post ( $p<0.05$ ). As for NGF, there was statistically significant difference in the treatment effect ( $p<0.05$ ), the period effect ( $p<0.01$ ) and the treatment-by-period interaction effect ( $p<0.01$ ). According to the post-hoc test, in the case of SOC condition there was a significant increase of NGF after treatment, and NGF concentration was significantly higher at Post, even at Post-2 h ( $p<0.05$ ), compared to CON condition. In contrast, in the case of CON condition there was no significant difference at every period (Pre, Post, Post-2 h), as in the case of BDNF.

It has been reported that regular exercise was effective not only for the prevention of metabolic diseases but also for the improvement of brain health and cognitive function [2,11,18]. Positive relationship between cardiovascular health and cognitive functions has been suggested, therefore, exercise which improves cardiovascular health is suggested to improve brain functions [39]. Also, it has been reported that a soccer game involves about 10 km of movement during the 90 minutes of the first and second halves, with 15 m sprint running every 90 seconds, elevating maximum plasma lactate concentration above 10 mmol/l [20]. On the other hand, Joe (1997) reported that jump motions for heading accounted for 9 % of the total movements during soccer game [20]. Based on such results of preceding researches, it is considered that the increased serum BDNF and NGF concentrations after the physical education activity of soccer in the present research were due to repeated movements during the soccer game, such as strenuous running, sprint running,

and jump motion for heading. Similar concrete results have been obtained by Winter *et al.* (2007), who reported a significant increase of serum BDNF in 27 healthy men after repeated high intensity sprint running at a plasma lactate concentration of 10 mmol/l [38]; and also by Bamaç *et al.* (2011), who reported an increase of serum BDNF and NGF concentrations after repeated heading motions [4]. The result of the present research is supported further by other researches reporting the increase of plasma BDNF and NGF concentrations in human after acute aerobic exercises of various intensities [12,13,42]. On the other hand, it has been reported that BDNF could pass through blood-brain barrier (BBB) even though its molecular weight is 27.0 kDa [28,30], and that there was a high correlation ( $r=0.81$ ) between serum BDNF concentration and cortical BDNF level in the rat [21]. With this result taken into consideration, it is believed that the peripheral BDNF increased by the physical education activity of soccer flowed into the brain to exert positive effect on brain health and neurocognitive function.

#### Changes in serum IGF-1 level

The changes of IGF-1 according to the soccer game were presented in Table 2. The result of two-way repeated ANOVA of IGF-1 showed statistically significant difference in the treatment effect ( $p<0.05$ ) and the treatment-by-period interaction effect ( $p<0.05$ ). According to the post-hoc test, in the case of SOC condition there was a significant increase of IGF-1 after treatment (Post) compared to before treatment (Pre), and significantly higher in the case of SOC condition compared to CON condition at Post ( $p<0.05$ ). In contrast, in the case of CON condition there was no significant difference at every period (Pre, Post, Post-2 h), as in the case of IGF-1.

Table 2. Comparison of neurotrophins and IGF-1

Variables	Treatment	Period			F value	p	
		Pre	Post	Post-2 h			
BDNF (pg/ml)	SOC	14576.00±2927.88 <sup>a</sup>	17365.67±2321.27 <sup>b,#</sup>	15480.67±2816.45 <sup>a</sup>	Treatment	1.339	0.267
	CON	14470.73±2999.07	14750.27±2351.22	14672.13±3449.18	Period	6.541	0.005 <sup>**</sup>
					T×P	4.046	0.029 <sup>*</sup>
NGF (pg/ml)	SOC	191.37±33.14 <sup>a</sup>	242.42±53.90 <sup>b,#</sup>	222.11±66.93 <sup>b</sup>	Treatment	8.420	0.012 <sup>*</sup>
	CON	186.75±23.90	191.25±30.80	182.52±33.56	Period	9.204	0.001 <sup>**</sup>
					T×P	6.347	0.005 <sup>**</sup>
IGF-1 (ng/ml)	SOC	291.00±33.51 <sup>a</sup>	325.93±32.01 <sup>b,#</sup>	310.13±47.03	Treatment	6.085	0.027 <sup>*</sup>
	CON	283.20±31.21	281.20±30.57	278.67±40.18	Period	2.733	0.082
					T×P	3.985	0.030 <sup>*</sup>

Values are means±SD, SOC: soccer game condition, CON: self-study condition, T×P: treatment-by-period interaction effect,

<sup>a,b</sup> Significantly different between letters ( $p<0.05$ ), <sup>#</sup> Significantly higher in SOC than CON ( $p<0.05$ ), <sup>\*</sup>  $p<0.05$ , <sup>\*\*</sup>  $p<0.01$

Such a result supports the results of preceding researches [3,6,33] that IGF-1 concentration was increased by various acute exercises. It has been reported by Antonelli *et al.* (2009) that acute exercise increased IGF-1 level in trained athletes[3]; and by Berg and Bang (2004) that circulating plasma IGF-1 concentration began to increase 5 minutes after the beginning of exercise [6]. A research by Roh (2010) also supports the result of the present research, who reported a significant increase of serum IGF-1 after treadmill running at 75% HRR intensity [33]. In addition, there are reports that a portion of the increased peripheral IGF-1 permeates through blood-brain barrier (BBB) to exert an effect on the increase of IGF-1 expression in the brain [32]; and that the artificial injection of IGF-1 into peripheral blood vessel caused the increase of BDNF expression in the hippocampus, as exercise caused such an increase [7]. Further, the prevention of the influx of IGF-1 into the brain by injecting the antibody against IGF-1 into peripheral blood vessel suppressed the protective effect of exercise against brain damage [8]. With all these results taken together into consideration, it is believed that IGF-1 is an important factor involved in the signal transduction pathway that regulates the expression of neurotrophins, including BDNF.

#### Changes in Stroop Color-Word test score

The changes of Stroop Color-Word test score according to the soccer game were presented in Table 3. The result of two-way repeated ANOVA of Word test score showed statistically significant difference in the period effect ( $p<0.05$ ), but in the case of Color test score there was no significant difference in the treatment effect, the period effect, and the treatment-by-period interaction effect. In con-

trast, Color-Word test score showed statistically significant difference in the treatment-by-period interaction effect ( $p<0.01$ ). According to the post-hoc test, in the case of SOC condition there was a significant increase of Color-Word test score after treatment (Post) compared to before treatment (Pre), and significantly higher in the case of SOC condition compared to CON condition at Post and Post-2 h ( $p<0.05$ ). In contrast, in the case of CON condition there was no significant difference at every period (Pre, Post, Post-2 h), as in the case of Color-Word test score.

This result supports the reports of the preceding researches that acute exercise could improve neurocognitive function as judged by the Stroop test of brain function [5,38]; and that proper exercise could positively promote human cognitive processes, such as memory, perception and problem-solving ability [17]. More concretely, Yanagisawa *et al.* (2010) reported that there was a significant improvement of Stroop test score after cycle ergometer exercise at 50%  $VO_{2peak}$  compared to before the exercise, and that the exercise elicited the increase of the activity of dorsolateral prefrontal cortex to improve cognitive function [41]. Barella *et al.* (2010) also reported that significantly higher Stroop test score was achieved by healthy elderly people who had been treated with 20 minutes of waking exercise, compared to the control group that had been seated to take a rest [5]. In addition, Gu *et al.* (1992) subdivided a group of teen agers into an exercise group (EG) and a control group (CG) to examine their memory, perception and problem-solving ability after exercise (in the case of EG), or after study or reading (in the case of CG), and the result showed that higher scores were achieved by EG in all the items. He suggested that such a result was due to the promotion of attentional process

Table 3. Comparison of Stroop Color-Word test score

Variables	Treatment	Period			F value	p	
		Pre	Post	Post-2 h			
Word test (score)	SOC	106.53±9.49	112.80±12.73	111.07±12.67	Treatment	4.179	0.060
	CON	106.13±9.61	106.67±10.95	104.47±8.94	Period	4.845	0.016*
					T×P	2.581	0.094
Color test (score)	SOC	97.67±9.03	104.33±11.67	103.00±14.12	Treatment	2.505	0.136
	CON	99.07±11.00	100.20±9.78	96.07±10.91	Period	1.623	0.215
					T×P	2.828	0.076
Color-Word test (score)	SOC	88.60±9.77 <sup>a</sup>	94.80±10.85 <sup>b,#</sup>	91.67±9.74 <sup>#</sup>	Treatment	2.777	0.118
	CON	90.20±12.89	89.93±11.80	86.40±11.92	Period	2.243	0.125
					T×P	5.698	0.008**

Values are means±SD, SOC: soccer game condition, CON: self-study condition, T×P: treatment-by-period interaction effect, <sup>a,b</sup> Significantly different between letters ( $p<0.05$ ), <sup>#</sup> Significantly higher in SOC than CON ( $p<0.05$ ), \* $p<0.05$ , \*\* $p<0.01$

by exercise, through the elevation of physical and mental arousal [17]. In line with such reports, the result of the present research showing a significant improvement of Stroop Color-Word test score after exercise is considered due to the increase of the activities of neurotrophins, such as BDNF and NGF, caused by the physical education activity of soccer. The present result is also supported by Ferris *et al.* (2007), who suggested that the increase of BDNF might be the major cause of the higher scores on the Word, Color, and Color-Word tests after acute exercise [12].

In conclusion, the physical education activity of soccer caused the significant increase of the concentrations of neurotrophins (BDNF, NGF) and IGF-1. Especially, IGF-1 is believed to be a mediating factor involved in the signal transduction pathway that induces the expression of neurotrophins such as BDNF. In addition, based on the Stroop Color-Word test score, it is considered that the increase of neurotrophins caused by exercise also exerts a positive effect on neurocognitive function.

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초록 : 1회성 축구활동이 남자 청소년의 혈청 neurotrophins 수준과 신경인지 기능에 미치는 영향

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본 연구의 목적은 1회성 축구활동이 남자 청소년의 혈청 뉴로트로핀 수준과 신경인지 기능에 미치는 영향을 규명하는데 있다. 연구의 대상은 건강한 남자 청소년 15명으로 하였으며, 연구 대상은 축구활동 처치(SOC) 조건과 자율학습 처치(CON) 조건의 2가지 처치 조건에 참여하였다. 채혈은 처치 전(Pre), 처치 후(Post), 처치 후 2시간(Post-2 h) 시점에서 총 3회 실시하였으며, 채취된 혈액을 이용하여 혈청 serum brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), insulin-like growth factor-1 (IGF-1)을 분석하였다. 신경인지 기능의 평가는 Stroop 색체-단어 검사를 이용하였으며, 채혈 시점과 동일한 시점에서 측정하였다. 연구 결과, 혈청 BDNF, NGF, IGF-1 수준은 축구활동 후 유의하게 증가하였으며( $p < 0.05$ ), Post 시점에서 SOC 조건이 CON 조건과 비교하여 유의하게 높게 나타났다( $p < 0.05$ ). Stroop 색체-단어 검사는 색체-단어 검사 점수에서 축구활동 후 유의한 증가를 나타내었으며( $p < 0.05$ ), Post 시점과 Post-2 h 시점에서 SOC 조건이 CON 조건과 비교하여 유의하게 높게 나타났다( $p < 0.05$ ). 이상의 결과를 종합하면, 1회성 축구활동이 뉴로트로핀을 증가시켜 신경인지 기능에 긍정적인 영향을 나타낸 것으로 판단된다.