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# Impact of exercise training on neuroplasticity-related growth factors in adolescents

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## Abstract

**Objectives:** We aimed to determine the effect of exercise training on plasma levels of brain-derived neurotrophic factor (BDNF), and serum insulin-like growth factor-1 (IGF-1) as well as cAMP response element-binding (CREB) activation in peripheral blood mononuclear cells (PBMCs) in adolescents. **Methods:** Nine trained and seven sedentary male adolescents, matched in age (14.0±2.2 years), were recruited for the study. Trained boys performed higher physical activity levels (expressed both as total energy expenditure and as physical activity energy expenditure) and showed significant bradycardia when compared with sedentary ones. **Results:** We found that BDNF and IGF-1 levels were significantly higher in trained adolescents than in sedentary ones. However, no effect of training was found in the activation of CREB in PBMCs. **Conclusions:** We demonstrated the increase of neuroplasticity-related proteins due to exercise training in adolescents. Our results emphasize the significance and impact of exercise in this developmental period.

**Keywords:** BDNF, IGF-1, CREB, Peripheral Blood Mononuclear Cells

## Introduction

Exercise training influences a wide range of cognitive processes, one of the principal pathways being through neurotrophic factors (NFs)<sup>1</sup>, which is a family of proteins responsible for growth, differentiation, and survival of neurons<sup>2</sup>. Brain derived neurotrophic factor (BDNF) is the most prevalent growth factor in the central nervous system and its modulation by (chronic or acute) exercise has been extensively described in both adult humans<sup>3</sup> and animals. Exercise increases BDNF levels not only in the brain, but also in blood and through its high-affinity receptor, tyrosine kinase type 2 (TrkB), it activates the cAMP response element-binding pro-

tein (CREB) transcription factor which is responsible for the hippocampal expression of several genes required for learning and memory<sup>4</sup>. Similarly, increased phosphorylated CREB (pCREB) has been reported in lymphocytes from depressive patients in response to antidepressant treatment<sup>5</sup>.

The use of peripheral blood mononuclear cells (PBMCs) has been recognized as an appropriate cellular model to investigate the possible effect of the activation of proplastic signal transduction cascades induced by exogenous stimuli<sup>6</sup>. However, to our knowledge, the effect of BDNF on CREB activation in PBMCs has never been investigated in exercise training studies. Moreover, insulin-like growth factor-1 (IGF-1) mediates the exercise-induced increase in BDNF, neurogenesis, and cognitive performance<sup>7</sup>.

Low levels of BDNF are found in patients with neurodegenerative diseases and different psychiatric disorders including depression, post-traumatic stress disorder, schizophrenia, obsessive-compulsive disorder, autism, bipolar disorder, addiction, and attention-deficit hyperactivity disorder. Moreover, several antidepressant, antipsychotic, and euthymic drugs, as well as electroconvulsive therapy and transcranial magnetic stimulation, have been found to increase BDNF levels<sup>8</sup>. As many neural programs that shape behavior become established during

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adolescence<sup>9</sup>, any lifestyle factor that increases BDNF and IGF-1 during this developmental period could play a role in the regulation and growth of neurons and in the prevention of different psychiatric and neurological disorders during childhood and adolescence. The aim of this work was to study whether exercise training causes an increase in NFs in childhood.

## Materials and methods

### Participants and data collection

Nine trained and seven sedentary male adolescents, matched in age ( $14.0 \pm 2.2$  years), were recruited for the study. All trained adolescents were cyclists and had participated in regular sport activities for an average of five training sessions and nineteen hours per week for more than three years. By contrast, the sedentary subjects had not taken part in any regular exercise other than the usual physical exercise program included in the educational curriculum which comprises sport activities for two hours, twice a week; volunteers who exceeded these limits were excluded from the study. All the participants were non-smokers, free from any known illnesses, and provided written informed consent prior to participation. This study complies with the World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. The experimental protocol was approved by the Committee on Ethics in Research of the Faculty of Medicine, University of Valencia, Spain.

### Biochemical measurements

Venous blood samples were taken in the morning between 9:00 a.m. and 10:30 a.m. after an overnight fast and following a fifteen-minute rest in a supine position. To avoid changes in blood parameters induced by physical performance, the cyclists did not train or compete for at least 24 hours before the blood extractions. All blood samples were collected in vacutainers from a superficial vein of the antecubital fossa. Plasma (EDTA) and serum were immediately separated ( $1,500 \times g$ , 15 minutes, RT) and stored at  $-20^\circ\text{C}$  until testing. Peripheral blood mononuclear cells were also isolated using Ficoll tubes (BD Vacutainer<sup>®</sup> CPT<sup>™</sup>). After washing the cells, the pellet was resuspended in lysis buffer (Hepes 20 mM, pH 7.4; NaCl 100 mM; TritonX100 1%; NaF 50 mM;  $\beta$ -glycerophosphate 10 mM; PMSF 1 mM; sodium ortovanadate and protease inhibitor cocktail), sonicated, centrifuged ( $13,000 \times g$ , 10 minutes at  $4^\circ\text{C}$ ) and stored at  $-20^\circ\text{C}$  until testing. The protein contents of the extracts were quantified in duplicate by using the Bradford method<sup>10</sup>, and BSA standard.

Plasma BDNF levels were measured using an ELISA kit (CYT306, ChemiKine TM, Millipore, Temecula, CA) following the manufacturer's instructions. Serum IGF-1 levels were measured by an automated chemiluminescent assay system (IMMULITE 2000<sup>®</sup>, Siemens, Diagnostic Products Corp., Los Angeles, CA). Hemoglobin (Hb), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), fasting blood glucose (FBG), and iron levels were also determined according to standard laboratory operating procedures.

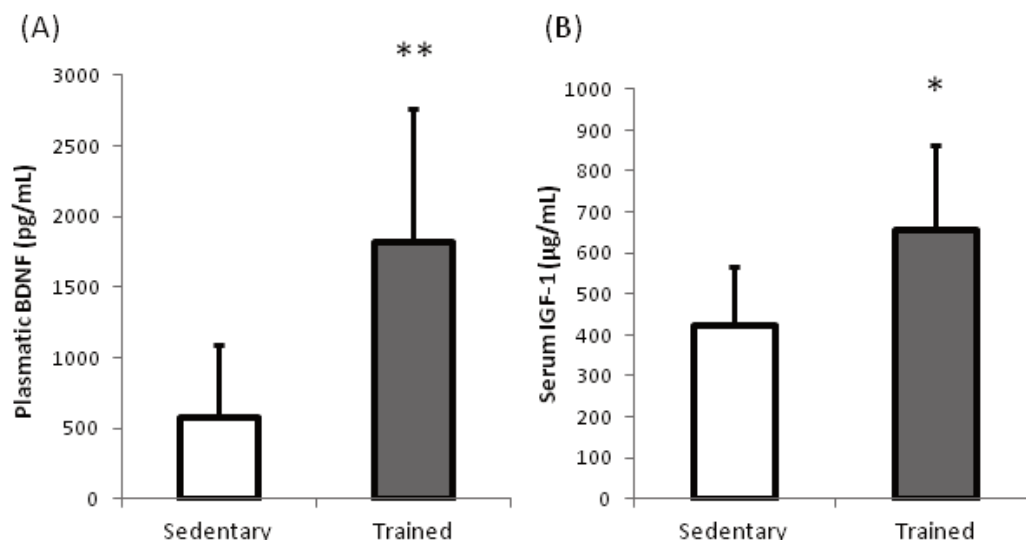
	Sedentary (n=7)	Trained (n=9)
Age (years)	13.4 $\pm$ 2.2	14.4 $\pm$ 2.1
Weight (kg)	47.4 $\pm$ 16.7	62.0 $\pm$ 8.5
Height (z-score)	0.3 $\pm$ 0.4	1.2 $\pm$ 1.0*
BMI (z-score)	-0.3 $\pm$ 1.2	0.7 $\pm$ 0.9
%Fat	18.1 $\pm$ 9.5	10.6 $\pm$ 1.8
%Lean mass	81.9 $\pm$ 9.5	89.4 $\pm$ 1.8
RHR (bpm)	70.7 $\pm$ 10.1	57.5 $\pm$ 6.4*
TC (mg/dl)	172.9 $\pm$ 29.4	145.1 $\pm$ 25.9
HDL-C (mg/dL)	68.6 $\pm$ 12.1	57.4 $\pm$ 8.4
LDL-C (mg/dL)	93.6 $\pm$ 27.6	86.1 $\pm$ 22.3
TG (mg/dL)	84.7 $\pm$ 52.8	61.8 $\pm$ 20.2
FBG (mg/dL)	90.7 $\pm$ 13.4	92.3 $\pm$ 8.3
Hb (g/dL)	14.0 $\pm$ 1.5	14.1 $\pm$ 1.0
Iron ( $\mu\text{g/dL}$ )	94.0 $\pm$ 32.6	95.6 $\pm$ 24.2
TEE (kcal/day)	1,951.7 $\pm$ 539.2	2,745.0 $\pm$ 411.0*
PAEE (kcal/day)	590.5 $\pm$ 122.1	945.3 $\pm$ 286.9*

**Table 1. Characteristics of the subjects.** Mean ( $\pm$ SD) results of BMI, body mass index; %Fat, percentage of body fat; %Lean mass, percentage of body lean mass; RHR, resting heart rate; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; FBG, fasting blood glucose; Hb, hemoglobin; TEE, total energy expenditure in a regular day without training session; PAEE, physical activity energy expenditure (over 3 METs) in a regular day without training session (\* $p < 0.05$ ).

CREB and pCREB were measured by Western blotting in PBMC lysates. The lysates (40  $\mu\text{g}$ ) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were then transferred to PVDF membranes, which were incubated overnight at  $4^\circ\text{C}$  with CREB and pCREB primary antibodies (1:800, Cell Signaling). Subsequently, the membranes were incubated in a secondary antibody for one hour at room temperature. One of the samples of each membrane was only incubated with the secondary antibody to guarantee specificity. Specific proteins were visualized by using the enhanced chemiluminescence procedure as specified by the manufacturer (Amersham Biosciences, Piscataway, NJ). Autoradiographic signals were assessed by using a scanning densitometer (BioRad, Hercules, CA). The protein levels of  $\alpha$ -tubulin (1:1,000, Sigma-Aldrich) were determined in all the experiments as a housekeeping protein marker. The determination of CREB activation was calculated by the following equation: pCREB/total CREB.

### Body composition and energy expenditure

In order to assess nutritional status, each child's weight, height, tricipital and subscapular skinfolds, using a standardized technique, and body mass index (BMI) calculations were performed. The z-score for age (based on the WHO Growth Reference, 2007), height, and BMI were calculated and the percentage of body fat (% fat) was recorded following the Slaughter equation<sup>11</sup>. The percentage of lean mass (% lean mass) was calculated by subtracting total weight from fat



**Figure 1. Peripheral neurotrophic factors in sedentary and trained adolescents.** Mean ( $\pm$ SD) results of BDNF plasma levels (A) and IGF-1 serum levels (B) of sedentary (n=7) and trained adolescents (n=9). (\*) indicates  $p<0.05$  and (\*\*) indicates  $p<0.01$  vs sedentary group.

weight. All subjects were monitored according to the pubertal development Tanner scale<sup>12</sup>.

The total energy expenditure (TEE) and physical activity energy expenditure (PAEE) were evaluated by calorimetry. For this purpose, the subjects wore a SenseWear Armband® (Body-Media) for three ordinary days (not including a training session) before the blood collection. Total energy expenditure was defined as the total kcal consumed per day, whereas PAEE was defined as the kcal consumed over three METs.

#### Statistical analysis

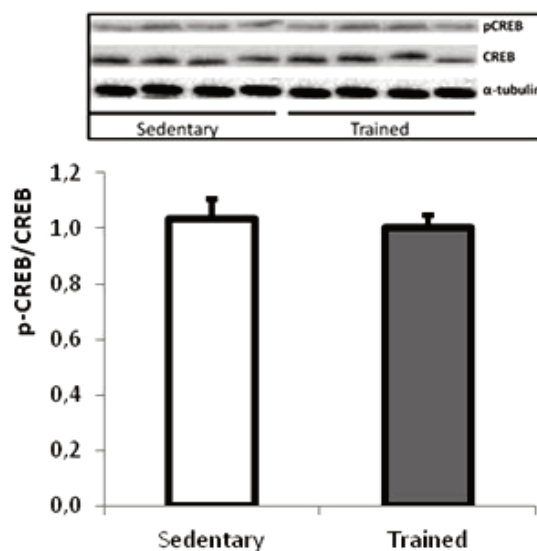
Data are shown as mean  $\pm$  standard deviation (SD). The comparisons between the sedentary and trained subjects were performed using the unpaired Student's *t*-test. Statistical significance was defined as  $p<0.05$ .

## Results

Table 1 shows the morphological and metabolic characteristics of the subjects. There were no differences in age, weight, BMI z-score, % fat, % lean mass, TC, HDL-C, LDL-C, TG, FBG, Hb, or free iron. However, statistically significant differences were observed in the z-score of height, resting heart rate (RHR), TEE, and PAEE between the sedentary and trained groups.

Figure 1A shows a significant increase in plasma BDNF levels in the trained adolescents when compared with the sedentary subjects (1,815.3 $\pm$ 948.6 pg/mL vs 570.7 $\pm$ 516.8 pg/mL). We also found a significant increase in serum IGF-1 levels in the trained adolescents when compared with the sedentary subjects (654.0 $\pm$ 207.5  $\mu$ g/mL vs 421.3 $\pm$ 146.8  $\mu$ g/mL) (Figure 1B).

No effect of the increase in peripheral BDNF induced by training was found in the activation of CREB in PBMCs (Figure 2).



**Figure 2. Activation of CREB in PBMCs.** Mean ( $\pm$ SD) results of CREB activation (pCREB/total CREB) in PBMCs of sedentary (n=7) and trained adolescents (n=9).

Consequently, this finding and our other results described above show that exercise training increases plasma BDNF and serum IGF-1 levels in adolescents.

## Discussion

It has been previously noted that exercise is involved in the maintenance of the synaptic structure<sup>4</sup>, axonal elongation<sup>13</sup>, and neurogenesis<sup>14</sup> through the induction of NFs in animal models<sup>4,7</sup>.

To our knowledge, this is the first report that investigates the effect of exercise training on peripheral NFs in healthy adolescents<sup>15</sup>. We found a significant increase in both the BDNF and IGF-1 levels in the trained subjects.

Although levels of BDNF and IGF-1 change according to age, body weight, and BMI<sup>16</sup>, in the current study, there were no significant differences in age and body-composition parameters between the two groups. It has been shown that peripheral BDNF levels can also be decreased in psychiatric and neurological disorders<sup>8</sup> but the medical doctors involved in this study did not detect any disorder in any of the boys we studied. Thus, the changes reported in NFs can be attributed to the exercise training itself.

The higher plasma levels of BDNF induced by exercise did not modify the CREB activation in PBMCs. One limitation of this work is the missing assessment of CREB activation in specific PBMCs sub-populations, because it is known that the percentage of individual cell populations may vary in response to exercise<sup>17</sup>. Therefore, although there is a release of BDNF in the different subtypes of PBMCs<sup>18-19</sup> the mechanism by which mononuclear cells activate CREB remains unclear. Even though *in vitro* experiments have demonstrated that exogenous BDNF is a potent inducer of CREB activation in T lymphocytes<sup>5</sup>, we did not observe this phenomenon *in vivo* in PBMCs.

The role of the most important NFs (BDNF and IGF-1) in adolescence, a critical period of central nervous system development, remained unclear. However, we have demonstrated the increase of these neuroplasticity-related proteins due to exercise training during this stage. Overall, our results emphasize the significance and impact of exercise in adolescence, when many neural programs that shape behavior become established.

We are aware of some limitations in our study; for instance, the sample size is small and this limitation may decrease the strength of our statistical analysis. Consequently, more research is needed to extend our conclusions.

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