



# Effect of Intensive Exercise Training and Vitamin E Supplementation on the Content of Rat Brain-Derived Neurotrophic Factors

Mohsen Foadoddini,<sup>1</sup> Mohammad Esmail Afzalpour,<sup>2</sup> Hossein TaheriChadorneshin,<sup>2</sup> and Seyed-Hosein Abtahi-Eivary<sup>3,\*</sup>

<sup>1</sup>Cardiovascular Diseases Research Center, Birjand University of Medical Sciences, Birjand, IR Iran

<sup>2</sup>Department of Sport Sciences, University of Birjand, Birjand, IR Iran

<sup>3</sup>Department of Biochemistry, Gonabad University of Medical Sciences, Gonabad, IR Iran

\*Corresponding author: Seyed-Hosein Abtahi-Eivary, Department of Biochemistry, School of Medicine, Gonabad University of Medical Sciences, Gonabad, IR Iran. Tel: +98-9155331265, Fax: +98-5157225027, E-mail: abtahi.h@gmu.ac.ir

Received 2017 July 02; Revised 2017 September 10; Accepted 2017 October 21.

## Abstract

**Background:** Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) and Tumor Necrosis Factor alpha (TNF-α) increase Brain-Derived Neurotrophic Factor (BDNF) and Glial Cell Line-Derived Neurotrophic Factor (GDNF) expression. Athletics use anti-oxidant and anti-inflammatory supplementations to reduce H<sub>2</sub>O<sub>2</sub> and TNF-α level. There is insufficient information about vitamin E supplementation on neurotrophic adaptations induced by intensive exercise training.

**Objectives:** The aim of the present study was to investigate the effects of intensive exercise training in combination with vitamin E supplementation on BDNF and GDNF content of rat brain.

**Methods:** In an experimental study in Iran, 40 male Wistar rats were randomly assigned to Control (C), Sham (S), Vitamin E (VTE), Exercise training (ET), Exercise training + Vitamin E (ET+VTE) groups, with 8 rats in each group. Exercise training on treadmill (27 m/minute) was carried out in combination with vitamin E supplementation (60 mg/kg/day) for six weeks. Statistical significance was determined at P < 0.05 using one-way analysis of variance (SPSS software, version 16.0).

**Results:** Vitamin E supplementation increased brain vitamin E levels in VTE and ET+VTE groups (P = 0.001). Significant increments in the levels of H<sub>2</sub>O<sub>2</sub> (P = 0.007), TNF-α, (P = 0.001), BDNF (P = 0.001), and GDNF (P = 0.001) in the ET group were shown; however, vitamin E supplementation could not change exercise-induced increases on the above-mentioned factors in the ET+VTE group (the p-values were equal to 0.024, 0.001, 0.001, and 0.001 respectively).

**Conclusion:** Intensive exercise training increases BDNF and GDNF contents in the whole brain of rats, yet vitamin E does not influence neurotrophic adaptations induced by intensive exercise training.

**Keywords:** Brain-Derived Neurotrophic Factor, Exercise, Glial Cell Line-Derived Neurotrophic Factor, Hydrogen Peroxide, Tumor Necrosis Factor Alpha, Vitamin E

## 1. Background

Neurotrophic factors are secretory proteins produced in different regions of the nervous system and initiate their functions by binding to their specific receptors (1,2). Brain-Derived Neurotrophic Factor (BDNF) is one of the most abundant and versatile neurotrophic factors, which by increase in neuroplasticity, neurogenesis, dendritogenesis, and synaptogenesis improves learning and recall of memory (2-4). Increasing anti-apoptotic protein, Glial Cell Line-Derived Neurotrophic Factor (GDNF) acts as the most potent survival factor for dopaminergic, cortical, and motor neurons, thereby, improves motor tasks (5-7).

It has been shown that Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) and Tumor Necrosis Factor-alpha (TNF-α) affect BDNF and

GDNF expression by increase in activation of some transcription factors, including Nuclear Factor-Kappa B (NF-κB) and cAMP Response Element Binding Protein (CREB) (7-10). An in-vitro study showed that H<sub>2</sub>O<sub>2</sub> increased GDNF protein in neuron-glia mixed cultures and protected dopaminergic neurons (7). In addition, 10-week H<sub>2</sub>O<sub>2</sub> injection increased GDNF contents in the cervical region of spinal cord in rats. However, 2 other studies suggested that N-acetyl-L-cysteine and N-tert-butyl-α-phenylnitron (PBN), as antioxidant, inhibited BDNF release from the microvascular endothelial cell line of the brain (9) and reduced BDNF levels in the cervical region of the spinal cord (1), respectively. Also, it has been shown that superoxide anion, induced by activation of NADPH oxidase, increases

BDNF expression by enhancing CREB phosphorylation (8). In contrast, inhibitors of NADPH oxidase, counteracting oxidative stress, reduced BDNF production in rostral ventrolateral medulla of rats by suppression of CREB (8). Tumor Necrosis Factor- $\alpha$  up-regulates BDNF and GDNF expression in brain astrocytes by activation of NF- $\kappa$ B (10). In addition, it has been shown that TNF $\alpha$  induces BDNF and GDNF expression in dorsal root ganglia (11). However, treatments antagonizing TNF- $\alpha$  action inhibited BDNF release from astrocytes (10) and reduced plasma BDNF by suppression of NF- $\kappa$ B activation (12). In addition, immune cells increased BDNF and its receptor in dorsal root ganglia by releasing pro-inflammatory cytokines (11).

Exercise training, especially at mild to moderate intensity, is a noninvasive approach to enhance neurotrophins at both the transcriptional and translational level in the brain (13). In this context, it has been shown that 8 weeks of swimming improves memory function through increase in BDNF content in the hippocampus (2). On the other hand, down-regulation of BDNF contents has been observed following detraining (2, 14). Pengyun et al. (2012) reported a coincident increase of NF- $\kappa$ B signaling and BDNF expression in hippocampus of rats following exhausted swimming exercise (15). Although long-term running on the treadmill increased BDNF in the hippocampus through increase in phosphorylation of CREB (16), short-term (3, 7 and 15 days) running on the treadmill (40 minutes, 10 m/minute) did not influence BDNF contents in the hippocampus (17). Also, exercise training prevents aging-induced decline of the BDNF level (16). In relation to other neurotrophins, such as GDNF, it has been established that an 18-week mild to moderate exercise training improves movement and coordination balance through increase in GDNF content in the striatum of mice with chronic Parkinson's disease (6). According to Groover et al. (2013), a 12-week moderate wheel running resulted in a 1.4-fold increase in GDNF contents in sciatic nerve and spinal cord (18). Furthermore, involuntary running elicited the greatest increase in GDNF protein content in the spinal cord compared with swimming and voluntary running (19). Collectively, studies suggest that low intensity exercise training increases BDNF and GDNF content in the brain (6, 13, 16, 18). In fact, there are exercises performed at high intensity and long duration that are associated with production of stress factors with potential adverse effects on BDNF and GDNF (1). However, there is little information regarding whether intense exercise could be harmful. In reality, the effects of intensive exercise training on BDNF and GDNF contents of the brain have not been well examined. The importance of this issue becomes even more evident considering that H<sub>2</sub>O<sub>2</sub> (20) and TNF- $\alpha$  (21) contents increase following intensive exercise. As men-

tion previously, H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$  increase neurotrophins expression in different regions of the nervous system (7-10). However, interactive effects of H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$  content on neurotrophin adaptations, following intense training, have not been well established.

Athletes spent high costs on anti-inflammatory and antioxidant supplements such as vitamin E to reduce inflammatory and reactive species following intensive exercise (22). It has been shown that vitamin E supplementation (5.0 g  $\alpha$ -tocopherol acetate/kg of food) could reduce the levels of H<sub>2</sub>O<sub>2</sub> in the brain (23, 24). Furthermore, it has been reported that vitamin E supplementation reduces TNF- $\alpha$  content in the hippocampus and frontal cortex by reducing microglial activation (25). It also decreases activation of transcription factors of NF- $\kappa$ B (26, 27) and CREB (28). Moreover, vitamin E succinate resulted in greater inhibition of NF- $\kappa$ B than other isoforms of vitamin E (27). It has also been shown that vitamin E has no effect on athletic performance (29) and the prevention and treatment of Alzheimer's disease or Parkinson's disease (30), however, even taking high doses of vitamin E may exacerbate diabetes and cardiovascular diseases (31). Due to the anti-inflammatory and antioxidant properties of vitamin E, it is necessary to accurately recognize whether there is an interaction between the use of vitamin E and neurotrophin contents following intensive exercise training in the brain. In reality, there is concern that vitamin E may interfere with adaptations resulting from exercise training.

## 2. Objectives

The aim of the present study was therefore to investigate how brain H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$  contents respond to intensive exercise training, how brain BDNF and GDNF contents respond to intensive exercise training and finally, how vitamin E affects BDNF and GDNF contents induced by intensive exercise training.

## 3. Materials and Methods

### 3.1. Animals

The present research was an experimental study. Forty male albino Wistar rats weighing 260 to 300 g (three months old) were housed in environmentally controlled rooms (22  $\pm$  2°C) with a 12-hour light-dark cycle. Rats had free access to standard rat food (Javaneh Khorasan Company, Iran) and tap water. After becoming familiarized with running on the treadmill, the animals based on simple randomization were assigned to Control (C), Sham (S), Vitamin E (V.TE), Exercise training (ET), Exercise training + Vitamin E (ET + V.TE) groups, with eight rats in each group.

All animal experiments conformed to the guidelines for the use and care of laboratory animals (NIH publication No. 86 - 23. Revised 1996) (32), and the study was approved by the ethics committee of Birjand University of Medical Sciences. Schematic timeline for the experimental protocol is shown in Figure 1.

### 3.2. Exercise Training Protocol

All animals were familiarized with running on a motor-driven treadmill (5 days, 10 minutes/day at 10 m/minute) [33]. Rats in the ET and ETS groups ran on the treadmill on the basis of overload principles for six weeks, six sessions per week at 27 m/minute. This intensity corresponded to 80% maximal oxygen uptake ( $VO_2\max$ ) (33). The duration of running in the first session was 20 minutes and increased by two minutes per day until 60 minutes was achieved by the 4<sup>th</sup> week. This intensity was maintained for the 2 following weeks (Table 1). At the beginning and the end of each session, warming-up and cooling-down periods were performed at 16 m/minute (33). Sedentary rat groups needed exposure to the treadmill except any running on the belt with turn off motor (6).

### 3.3. Vitamin E Preparation and Supplementation

Vitamin E succinate (Sigma-Aldrich, MO, USA) was dissolved in sesame oil purchased from the local market (60 mg/mL) (34). Rats in the S and ETS groups were supplemented orally with vitamin E (60 mg/kg body weight) for 6 days/week, 3 hours before exercise training (34, 35). Also, rats in the V group were supplemented orally by sesame oil (1 mL/kg of body weight) for 6 days/week. Sesame oil did not influence BDNF content in the hippocampus, amygdala, hypothalamus, and plasma corticosterone concentrations in rats (16, 36).

### 3.4. Tissue Preparation and Biochemical Assays

Rats were sacrificed 48 hours after the last exercise session by decapitation under anesthesia (Ketamine, 80 mg/kg and Xylazine, 8 mg/kg; IP prepared from Alfasan, Holland) (33) and the whole brain was removed (6). Despite different distribution of GDNF (7) and BDNF (37) levels in the brain, since small regions, such as the hippocampus and striatum, are not large enough to permit measurements, the whole brain was selected for the biochemical assays (4). To obtain a homogenized tissue sample, each brain was dipped to liquid nitrogen and smashed to a fine powder (5). Then, 1x phosphate buffered saline containing protease inhibitor cocktail (ProBlock™, Goldbio technology CO., USA) was added to micro-tube containing certain amounts of powdered brain. Commercial Enzyme Linked Immunosorbent Assay (ELISA) kits were used

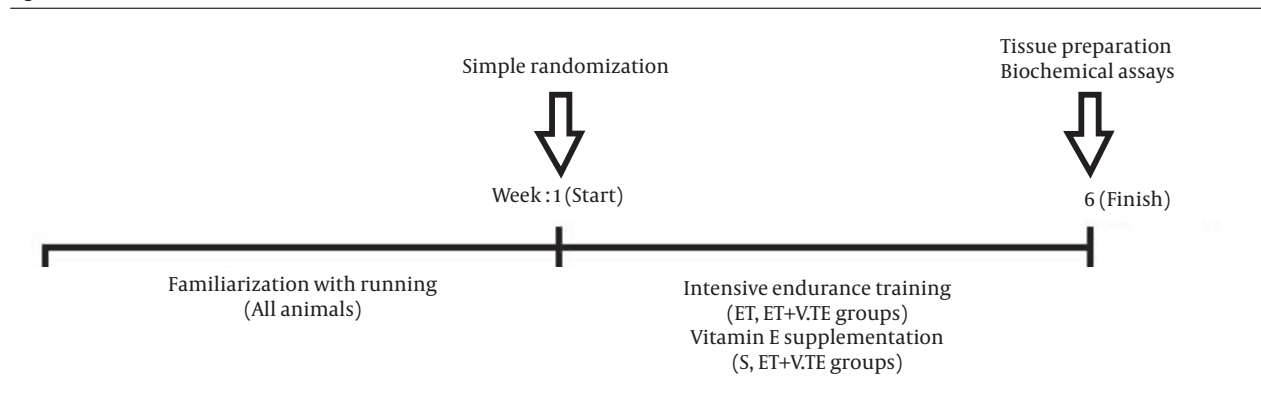
**Table 1.** Intensive Endurance Trainings Protocols

Week	Day	Intensive Endurance Training
Week 1	1	20 min, 27 m/min
	2	22 min, 27 m/min
	3	24 min, 27 m/min
	4	26 min, 27 m/min
	5	28 min, 27 m/min
	6	30 min, 27 m/min
Week 2	1	32 min, 27 m/min
	2	34 min, 27 m/min
	3	36 min, 27 m/min
	4	38 min, 27 m/min
	5	40 min, 27 m/min
	6	42 min, 27 m/min
Week 3	1	44 min, 27 m/min
	2	46 min, 27 m/min
	3	48 min, 27 m/min
	4	50 min, 27 m/min
	5	52 min, 27 m/min
	6	54 min, 27 m/min
Week 4	1	56 min, 27 m/min
	2	58 min, 27 m/min
	3	60 min, 27 m/min
	4	60 min, 27 m/min
	5	60 min, 27 m/min
	6	60 min, 27 m/min
Week 5 - 6	1 - 12	60 min, 27 m/min, to end of 6 <sup>th</sup> week

to measure total GDNF (#CSB-E04566r, Cusabio Biotech CO., LTD. Sino-American), total BDNF (#CSB-E04504r, Cusabio Biotech CO., LTD. Sino-American), and total TNF- $\alpha$  (#865.000.096, Diaclone SAS., France) contents. H<sub>2</sub>O<sub>2</sub> and vitamin E assays were carried out by H<sub>2</sub>O<sub>2</sub> colorimetric assay kit (#BC05-96, Biocore Diagnostik Ulm, German) and vitamin E colorimetric assay kit (#BC09-96, Biocore Diagnostik Ulm, German), respectively. The assays were carried out, according to the manufacturer's instructions and the optical density of samples was read using the ELISA reader (Biotek, USA), and the values were expressed based on tissue weight.

### 3.5. Statistical Analysis

Data were analyzed by Statistical Package for Social Sciences (SPSS Inc., Chicago, USA) software, version 16.0. All the data are presented as means  $\pm$  Standard Deviation

**Figure 1.** Protocol Timeline

After familiarized with running on treadmill (5 days, 10 min/day at 10 m/min), the animals based on simple randomization were assigned to Control (C), Sham (S), Vitamin E (V.TE), Exercise training (ET), Exercise training + Vitamin E (ET + V.TE) groups. Then, exercise training (six sessions per week at 27 m/min) and vitamin E supplementation (60 mg/kg body weight) were carried out for 6 weeks. Finally, whole brain of each rat was removed and selected for the biochemical assays.

**Table 2.** Means and Standard Deviations (M  $\pm$  SD) of Dependent Variables

Groups	Vitamin E ( $\mu\text{g/g}$ Tissue Weight)	H <sub>2</sub> O <sub>2</sub> ( $\mu\text{M/mg}$ Tissue Weight)	TNF- $\alpha$ ( $\text{pg/mg}$ Tissue Weight)	BDNF ( $\text{pg/mg}$ Tissue Weight)	GDNF ( $\text{pg/mg}$ Tissue Weight)
C	14.23 $\pm$ 4.10	0.59 $\pm$ 0.10	1.53 $\pm$ 0.24	13.58 $\pm$ 1.46	9.87 $\pm$ 1.27
S	17.20 $\pm$ 3.11	0.56 $\pm$ 0.15	1.57 $\pm$ 0.22	13.52 $\pm$ 1.33	9.9 $\pm$ 1.54
V.TE	33.83 $\pm$ 2.92	0.45 $\pm$ 0.09	1.22 $\pm$ 0.21	11.37 $\pm$ 1.24	7.48 $\pm$ 1.64
ET	12.10 $\pm$ 4.05 <sup>a</sup>	0.81 $\pm$ 0.16 <sup>a</sup>	2.82 $\pm$ 0.36 <sup>a</sup>	24.02 $\pm$ 4.27 <sup>a</sup>	17.1 $\pm$ 3.33 <sup>a</sup>
ET + V.TE	27.44 $\pm$ 1.41 <sup>a</sup>	0.78 $\pm$ 0.06 <sup>a</sup>	2.52 $\pm$ 0.18 <sup>a</sup>	21.91 $\pm$ 1.92 <sup>a</sup>	14.72 $\pm$ 2.23 <sup>a</sup>

Abbreviations: BDNF, Brain-Derived Neurotrophic Factor; C, Control; GDNF, Cell Line-Derived Neurotrophic Factor; S, Sham; V.TE, Vitamin E; ET, Exercise training; ET + V.TE, Exercise training + Vitamin E.

<sup>a</sup> Denotes a significant difference compared with C group.

(SD). After performing a test of normality by Shapiro-Wilk's test, statistical significance was determined at  $P < 0.05$  using one-way Analysis of Variance (ANOVA), followed by Bonferroni post-hoc comparison, to test the differences between groups. In addition, Pearson's correlation coefficient was calculated to determine the relationship between variables.

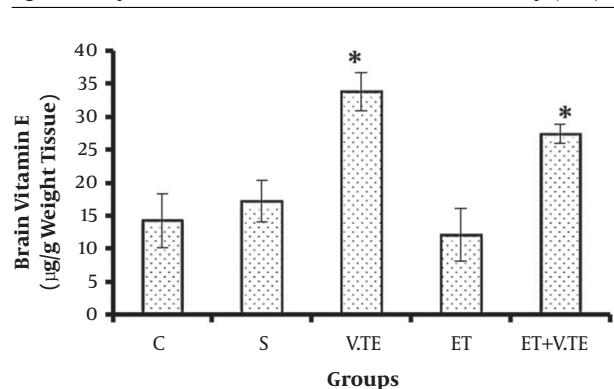
## 4. Results

### 4.1. Vitamin E levels

The results indicated that vitamin E supplementation increased brain vitamin E levels in V.TE ( $P = 0.001$ ) and ET + V.TE ( $P = 0.001$ ) groups compared with the C group (Figure 2). Means and standard deviations of vitamin E are presented in Table 2.

### 4.2. H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$ Contents

Intensive exercise training significantly increased brain H<sub>2</sub>O<sub>2</sub> levels in ET ( $P = 0.007$ ) and ET + V.TE ( $P = 0.024$ )

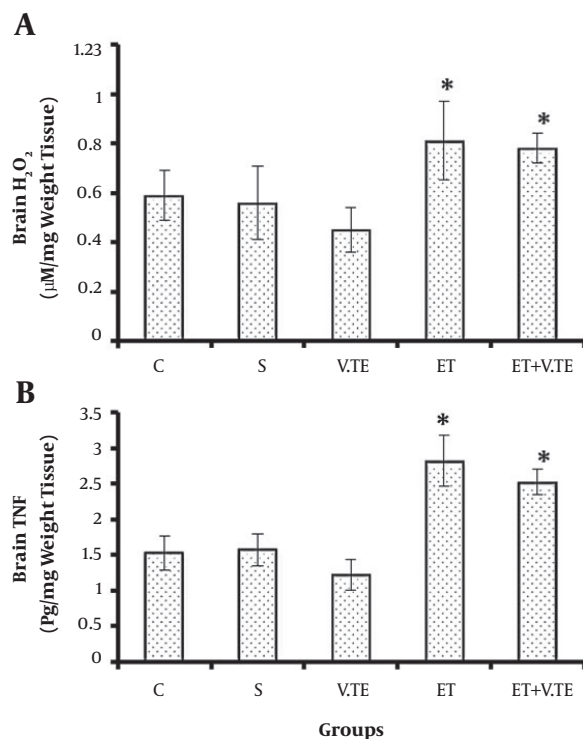
**Figure 2.** Comparison Vitamin E Levels in Whole Brain of Different Groups (n = 8)

Vitamin E supplementation increased vitamin E levels of whole brain in V.TE ( $P = 0.001$ ) and ET + V.TE ( $P = 0.001$ ) groups. The asterisk (\*) denotes a significant difference compared with C group. Abbreviations: C, Control; S, Sham; V.TE, Vitamin E; ET, Exercise training; ET + V.TE, Exercise training + Vitamin E.

groups compared with the C group. However, vitamin E supplementation could not influence brain H<sub>2</sub>O<sub>2</sub> levels in

V.TE ( $P = 0.593$ ) and ET + V.TE ( $P = 0.999$ ) groups (Figure 3A). In contrast, inflammatory marker, brain TNF- $\alpha$  contents, significantly increased in ET ( $P = 0.001$ ) and ET + V.TE ( $P = 0.001$ ) groups compared with the C group. However, vitamin E supplementation could not influence brain TNF- $\alpha$  contents in V.TE ( $P = 0.465$ ) and ET + V.TE ( $P = 0.522$ ) groups (Figure 3B). Means and standard deviations of H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$  are presented in Table 2.

**Figure 3.** Comparison of H<sub>2</sub>O<sub>2</sub> (A) and TNF- $\alpha$  (B) Contents in Whole Brain of Different Groups (n = 8)



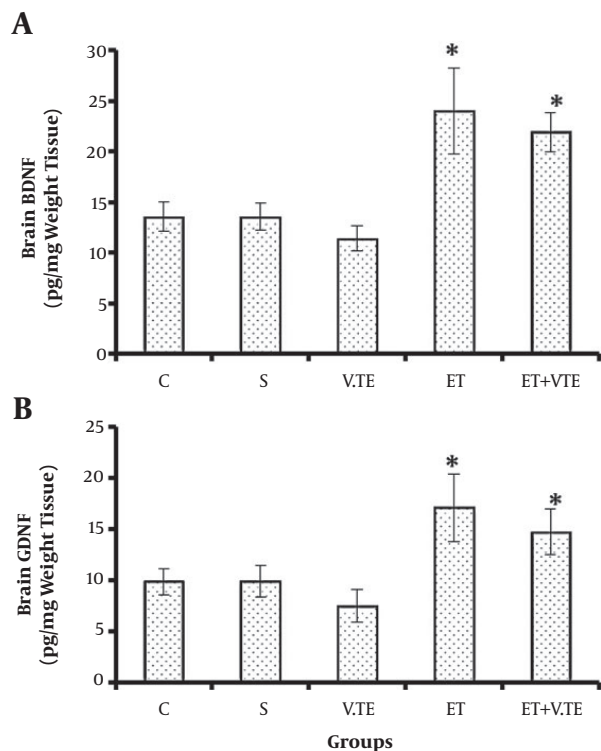
The levels of H<sub>2</sub>O<sub>2</sub> in whole brain was increased in ET ( $P = 0.007$ ) and ETS ( $P = 0.024$ ) groups. The protein content of TNF- $\alpha$  in whole brain was increased in ET ( $P = 0.001$ ) and ETS ( $P = 0.001$ ) groups. The asterisk (\*) denotes a significant difference compared with C group. Abbreviations: C, Control; S, Sham; V.TE, Vitamin E; ET, Exercise training; ET + V.TE, Exercise training + Vitamin E.

#### 4.3. BDNF and GDNF Contents

Brain BDNF content was significantly enhanced in ET ( $P = 0.001$ ) and ET + V.TE ( $P = 0.001$ ) groups compared with the C group. In contrast, vitamin E supplementation could not influence brain BDNF contents in V.TE ( $P = 0.462$ ) and ET + V.TE ( $P = 0.499$ ) groups (Figure 4A). In addition, brain GDNF content significantly enhanced in the ET ( $P = 0.001$ ) and ET + V.TE ( $P = 0.001$ ) groups compared with the C group. However, vitamin E supplementation could not influence brain GDNF contents in V.TE ( $P = 0.478$ ) and ET + V.TE ( $P =$

$0.493$ ) groups (Figure 4B). Means and standard deviations of BDNF and GDNF are presented in Table 2.

**Figure 4.** Comparison of BDNF (A) and GDNF (B) Contents in Whole Brain of Different Groups (n = 8)



The protein contents of BDNF and GDNF in whole brain were increased in ET ( $P = 0.001$ ) and ETS ( $P = 0.001$ ) groups. The asterisk (\*) denotes a significant difference compare with C group. Abbreviations: C, Control; S, Sham; V.TE, Vitamin E; ET, Exercise training; ET + V.TE, Exercise training + Vitamin E.

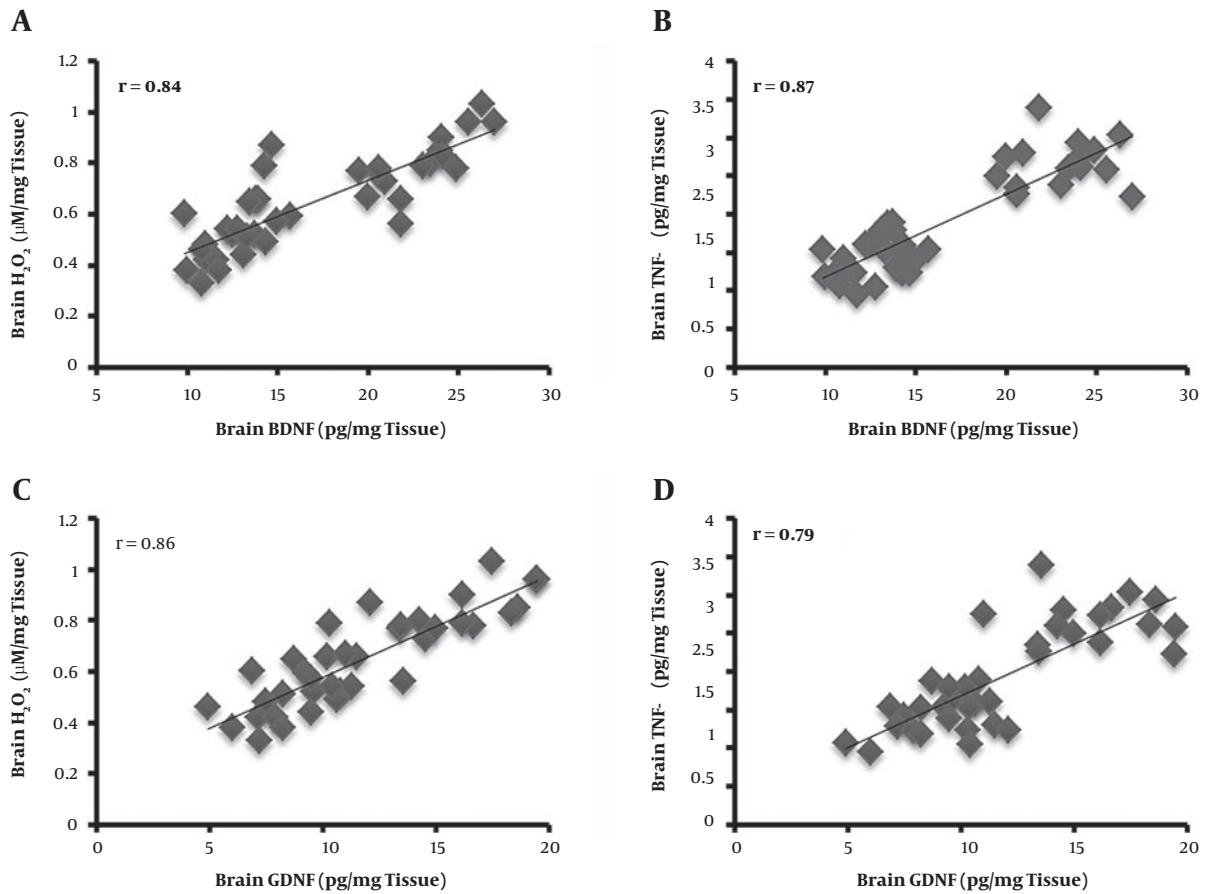
#### 4.4. Relationship Between Neurotrophins, Oxidative Stress, and Inflammatory Markers

Furthermore, the current results showed a significant positive correlation between BDNF with H<sub>2</sub>O<sub>2</sub> ( $r = 0.84$ ,  $P = 0.001$ ) and with TNF- $\alpha$  ( $r = 0.87$ ,  $P = 0.001$ ) (Figure 5A, 5B) and also between GDNF with H<sub>2</sub>O<sub>2</sub> ( $r = 0.86$ ,  $P = 0.001$ ) and with TNF- $\alpha$  ( $r = 0.79$ ,  $P = 0.001$ ) (Figure 5C, 5D).

## 5. Discussion

Various approaches have been used to increase neurotrophins in the brain. However, exercise training accounts for a non-pharmacological approach to maintain health and improve brain function. This study showed that intensive exercise training increased BDNF and GDNF contents in the brain and also there were positive correlations between the contents of above-mentioned factors



**Figure 5.** Correlation Between BDNF With H<sub>2</sub>O<sub>2</sub> (A) and With TNF $\alpha$  (B) in Whole Brain of Rats

In addition, correlation between GDNF with H<sub>2</sub>O<sub>2</sub> (A) and with TNF $\alpha$  (B) in whole brain of rats. The correlations were significant ( $P = 0.001$ ).

and H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$  levels. In addition, other studies have shown that exercise trainings with low to moderate intensity enhanced motor and cognitive processes through increase in neurotrophins (2, 6), which may have a protective role in various neurological diseases. Also, the results of this study showed that vitamin E supplementation did not influence neurotrophins adaptations, induced by exercise training in the brain.

Furthermore, BDNF and GDNF are 14- and 15-kDa monomer proteins synthesized and released by neurons, oligodendrocytes, glia cells, astroglia, microglia, satellite cells, schwann cells, stem cells, and blood cells (1, 19). In agreement with other studies, in which BDNF and GDNF levels increased in special regions of the nerve system, following exercise training with low to moderate intensity (7, 19), the current study showed increased contents of these neurotrophic factors in the entire brain following intensive exercise training, which may be due to alterations in

the synthesis and release from the above-mentioned cells. In other words, the current results are consistent with studies that used protocols with low to moderate intensity. However, it has been reported that short-term running on the treadmill does not influence BDNF (17) and GDNF (38) contents in the hippocampus and striatum, respectively. In addition, lack of changes in the basal forebrain BDNF content following 7 weeks of running on the treadmill has been attributed to learning, induced by the Morris maze task in the last week of training in both control and exercise training groups (39).

Increased levels of insulin-like growth factor 1 (40) and estrogen (16), and decreased levels of leptin (3) and corticosterone (16) are considered as potential mechanisms by which exercise training with low to moderate intensity increases neurotrophins. Similar to the study performed by TaheriChadorneshin et al. (33), the present study was intensive and resulted in enhancement of H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$  pro-

tein in the brain as reported by others (21). However, lack of changes in the TNF- $\alpha$  content in the entire brain and hypothalamus has been attributed to short term (41) and low intensity exercise training (42), respectively. Also, exercise training with low intensity did not influence the production of H<sub>2</sub>O<sub>2</sub> in the hippocampus and cerebellum (20). As shown previously, there are positive correlations between both H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$  levels with neurotrophic factors; in one study, it was shown that increase in whole brain BDNF content attributed to inflammation induced by exercise training (4). Also, it was reported that oxidative stress induced by exercise training influences neurotrophins adaptations in the cervical region of rats and there is a positive correlation between free radical concentration and BDNF contents (1). In addition, the levels of H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$  influence BDNF and GDNF expression by increase in translocation of p65: p50 of NF- $\kappa$ B complex from cytoplasm to nucleus (7, 10), and up-regulate neurotrophins expression via activation of CREB (8, 10). Overall, positive correlations between neurotrophins levels and contents of oxidative stress and pro-inflammatory factors indicate that changes in brain BDNF and GDNF after intensive exercise training, along with other possible factors, may be attributed to increase in oxidative stress and inflammation.

In agreement with other studies (24, 43), the current results have shown that vitamin E supplementation increased whole brain vitamin E levels to 1.37 times and 92% in S and ETS groups, respectively. Based on Ibrahim's study, in which vitamin E levels increased in a dose-dependent manner in the brain (44), Jolitha et al. reported an increase of 46% and 52% in vitamin E levels in hippocampus and cerebral cortex of rats following low dose supplementation of vitamin E (20). Furthermore, vitamin E supplementation increased vitamin E levels by 17% to 44% in the left and right ventricles of the heart of rats (45). This means that dosage of vitamin E, tissue-responses, and amount of fat in tissue may influence vitamin E uptake (43). The transfer of vitamin E from plasma to the cerebrospinal fluid and to the nerve cells is mediated by both apolipoprotein E (46) and scavenger receptor class B type I (47), which play decisive roles in vitamin E uptake by different regions of the brain. The current results showed that vigorous exercise did not change vitamin E levels in the brain. Similarly, acute exhaustive exercise and an 8-week exercise training on the treadmill had no effect on  $\alpha$ -tocopherol levels in the rat brain (48).

Vitamin E is used due to its antioxidant and anti-inflammatory properties. Vitamin E reduces H<sub>2</sub>O<sub>2</sub> levels through directly scavenging superoxide radicals or indirectly decreasing expression (48, 49) and activity of superoxide dismutase (50). Also, vitamin E reduces expression of TNF- $\alpha$  and inflammatory conditions by reducing NF- $\kappa$ B ac-

tivation (25, 26). Despite that vitamin E supplementation increased levels of vitamin E in the brain, it had no significant effect on H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$  content. This is consistent with some studies (20, 23) and inconsistent with others (24, 26). These discrepancies may be related to high doses of supplementation of  $\alpha$ -tocopherol acetate (2.0 or 5.0 g/kg of food) (23, 24) and long term supplementation of vitamin E (3 months) (23, 24, 26).

It has been reported that oxidative stress reduces BDNF content by increasing damage to cells and protein carbonyl and 8-Hydroxy-2'-deoxyguanosine levels. Thus, increases of neurotrophins by vitamin E, which has protective and survival effects on the cells, are expected. Vitamin E reduces oxidative damage in different regions of the brain (22). Therefore, the reason that vitamin E supplementation had no effect on BDNF and GDNF contents might be due to low dose of vitamin E supplementation, which did not allow vitamin E to reduce H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$  levels. However, vitamin E supplementation kept mRNA and protein levels of BDNF at base line levels in the hippocampus by reducing oxidative products (28). Vitamin E succinate resulted in greater inhibition of the NF- $\kappa$ B than  $\alpha$ -tocopherol and  $\alpha$ -tocopherol acetate. It seems likely that vitamin E compounds influence BDNF and GDNF contents differently (27). The brain consists of several regions that are different in their contributions to neurotrophins production (7, 37) and their vitamin E uptake from cerebrospinal fluid (47). In this context, it has been reported that hippocampus, midbrain, striatum, and frontal cortex have, in order, larger ventricular surface areas in contact with cerebrospinal fluid, resulting in up-taking of more vitamin E (47). Thus, the accumulation of vitamin E in the hippocampus is higher than other regions of the brain (47). Furthermore, BDNF and GDNF contents are higher in the hippocampus (37) and striatum (7), respectively. Thus, the other reason for the lack of significant effect of vitamin E on BDNF content may be due to determination of this factor in the entire brain instead of specific regions. In addition, it has been shown that vitamin E uptake and its concentration in the striatum is lower than other regions of the brain (43). It appears that vitamin E does not affect the GDNF content of striatum and the slight decrease of GDNF observed in this study might be caused by other regions of the brain. Hence, investigation of the effect of vitamin E supplementation on neurotrophins contents in specific regions of the brain is suggested for future researches.

### 5.1. Conclusions

Intensive exercise training increased GDNF and BDNF contents in the brain and these changes may be associated with H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$  enhancement following intensive exercise training. Also, vitamin E succinate sup-

plementations for 6 weeks did not appear to influence neurotrophins adaptations induced by intensive exercise training.

## 5.2. Limitations

The main limitation of the present study was that neurotrophins were measured in the whole brain instead of special regions of the brain. As previously mentioned, vitamin E uptake differs between various regions of the brain, which may differently influence neurotrophins content. Hence, investigating vitamin E supplementation effects on neurotrophins content in specific regions of the brain are recommended for future researches.

## Acknowledgments

The authors thank the staff of the biochemical laboratory of Gonabad University of Medical Sciences for their valuable assistance in carrying out the biochemical assays and the Animal Laboratory Center of Birjand University of Medical Sciences for their kind help.

## References

- Siamilis S, Jakus J, Nyakas C, Costa A, Mihalik B, Falus A, et al. The effect of exercise and oxidant-antioxidant intervention on the levels of neurotrophins and free radicals in spinal cord of rats. *Spinal Cord*. 2009;**47**(6):453-7. doi: [10.1038/sc.2008.125](https://doi.org/10.1038/sc.2008.125). [PubMed: [18936770](https://pubmed.ncbi.nlm.nih.gov/18936770/)].
- Radak Z, Toldy A, Szabo Z, Siamilis S, Nyakas C, Silye G, et al. The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. *Neurochem Int*. 2006;**49**(4):387-92. doi: [10.1016/j.neuint.2006.02.004](https://doi.org/10.1016/j.neuint.2006.02.004). [PubMed: [16564605](https://pubmed.ncbi.nlm.nih.gov/16564605/)].
- Seifert T, Brassard P, Wissenberg M, Rasmussen P, Nordby P, Stal-knecht B, et al. Endurance training enhances BDNF release from the human brain. *Am J Physiol Regul Integr Comp Physiol*. 2010;**298**(2):R372-7. doi: [10.1152/ajpregu.00525.2009](https://doi.org/10.1152/ajpregu.00525.2009). [PubMed: [19923361](https://pubmed.ncbi.nlm.nih.gov/19923361/)].
- Ogonovszky H, Berkes I, Kumagai S, Kaneko T, Tahara S, Goto S, et al. The effects of moderate, strenuous- and over-training on oxidative stress markers, DNA repair, and memory, in rat brain. *Neurochem Int*. 2005;**46**(8):635-40. doi: [10.1016/j.neuint.2005.02.009](https://doi.org/10.1016/j.neuint.2005.02.009). [PubMed: [15863241](https://pubmed.ncbi.nlm.nih.gov/15863241/)].
- McCullough MJ, Peplinski NG, Kinnell KR, Spitsbergen JM. Glial cell line-derived neurotrophic factor protein content in rat skeletal muscle is altered by increased physical activity in vivo and in vitro. *Neuroscience*. 2011;**174**:234-44. doi: [10.1016/j.neuroscience.2010.11.016](https://doi.org/10.1016/j.neuroscience.2010.11.016). [PubMed: [21081155](https://pubmed.ncbi.nlm.nih.gov/21081155/)].
- Lau YS, Patki G, Das-Panja K, Le WD, Ahmad SO. Neuroprotective effects and mechanisms of exercise in a chronic mouse model of Parkinson's disease with moderate neurodegeneration. *Eur J Neurosci*. 2011;**33**(7):1264-74. doi: [10.1111/j.1460-9568.2011.07626.x](https://doi.org/10.1111/j.1460-9568.2011.07626.x). [PubMed: [21375602](https://pubmed.ncbi.nlm.nih.gov/21375602/)].
- Saavedra A, Baltazar G, Duarte EP. Driving GDNF expression: the green and the red traffic lights. *Prog Neurobiol*. 2008;**86**(3):186-215. doi: [10.1016/j.pneurobio.2008.09.006](https://doi.org/10.1016/j.pneurobio.2008.09.006). [PubMed: [18824211](https://pubmed.ncbi.nlm.nih.gov/18824211/)].
- Chan SH, Wu CW, Chang AY, Hsu KS, Chan JY. Transcriptional upregulation of brain-derived neurotrophic factor in rostral ventrolateral medulla by angiotensin II: significance in superoxide homeostasis and neural regulation of arterial pressure. *Circ Res*. 2010;**107**(9):1127-39. doi: [10.1161/CIRCRESAHA.110.225573](https://doi.org/10.1161/CIRCRESAHA.110.225573). [PubMed: [20814019](https://pubmed.ncbi.nlm.nih.gov/20814019/)].
- Wang H, Ward N, Boswell M, Katz DM. Secretion of brain-derived neurotrophic factor from brain microvascular endothelial cells. *Eur J Neurosci*. 2006;**23**(6):1665-70. doi: [10.1111/j.1460-9568.2006.04682.x](https://doi.org/10.1111/j.1460-9568.2006.04682.x). [PubMed: [16553631](https://pubmed.ncbi.nlm.nih.gov/16553631/)].
- Saha RN, Liu X, Pahan K. Up-regulation of BDNF in astrocytes by TNF-alpha: a case for the neuroprotective role of cytokine. *J Neuroimmune Pharmacol*. 2006;**1**(3):212-22. doi: [10.1007/s11481-006-9020-8](https://doi.org/10.1007/s11481-006-9020-8). [PubMed: [18040799](https://pubmed.ncbi.nlm.nih.gov/18040799/)].
- Lin YT, Ro LS, Wang HL, Chen JC. Up-regulation of dorsal root ganglia BDNF and trkB receptor in inflammatory pain: an in vivo and in vitro study. *J Neuroinflammation*. 2011;**8**:126. doi: [10.1186/1742-2094-8-126](https://doi.org/10.1186/1742-2094-8-126). [PubMed: [21958434](https://pubmed.ncbi.nlm.nih.gov/21958434/)].
- Grimsholm O, Rantapaa-Dahlqvist S, Dalen T, Forsgren S. BDNF in RA: downregulated in plasma following anti-TNF treatment but no correlation with inflammatory parameters. *Clin Rheumatol*. 2008;**27**(10):1289-97. doi: [10.1007/s10067-008-0910-4](https://doi.org/10.1007/s10067-008-0910-4). [PubMed: [18484150](https://pubmed.ncbi.nlm.nih.gov/18484150/)].
- Berchtold NC, Chinn G, Chou M, Kesslak JP, Cotman CW. Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. *Neuroscience*. 2005;**133**(3):853-61. doi: [10.1016/j.neuroscience.2005.03.026](https://doi.org/10.1016/j.neuroscience.2005.03.026). [PubMed: [15896913](https://pubmed.ncbi.nlm.nih.gov/15896913/)].
- Berchtold NC, Castello N, Cotman CW. Exercise and time-dependent benefits to learning and memory. *Neuroscience*. 2010;**167**(3):588-97. doi: [10.1016/j.neuroscience.2010.02.050](https://doi.org/10.1016/j.neuroscience.2010.02.050). [PubMed: [20219647](https://pubmed.ncbi.nlm.nih.gov/20219647/)].
- Pengyun Z, Sheng H, Ruiqing Z, Shaojun Q, Guijiao Y. Expressional change of NF-κB and BDNF in hippocampus of rats after exhausted exercise. *Chines J Neuroanatom*. 2012;(1).
- Marosi K, Felszeghy K, Mehra RD, Radak Z, Nyakas C. Are the neuroprotective effects of estradiol and physical exercise comparable during ageing in female rats? *Biogerontology*. 2012;**13**(4):413-27. doi: [10.1007/s10522-012-9386-3](https://doi.org/10.1007/s10522-012-9386-3). [PubMed: [22722983](https://pubmed.ncbi.nlm.nih.gov/22722983/)].
- Ferreira AF, Real CC, Rodrigues AC, Alves AS, Britto LR. Short-term, moderate exercise is capable of inducing structural, BDNF-independent hippocampal plasticity. *Brain Res*. 2011;**1425**:111-22. doi: [10.1016/j.brainres.2011.10.004](https://doi.org/10.1016/j.brainres.2011.10.004). [PubMed: [22035567](https://pubmed.ncbi.nlm.nih.gov/22035567/)].
- Groover AL, Ryals JM, Guilford BL, Wilson NM, Christianson JA, Wright DE. Exercise-mediated improvements in painful neuropathy associated with prediabetes in mice. *Pain*. 2013;**154**(12):2658-67. doi: [10.1016/j.pain.2013.07.052](https://doi.org/10.1016/j.pain.2013.07.052). [PubMed: [23932909](https://pubmed.ncbi.nlm.nih.gov/23932909/)].
- McCullough MJ, Gyorkos AM, Spitsbergen JM. Short-term exercise increases GDNF protein levels in the spinal cord of young and old rats. *Neuroscience*. 2013;**240**:258-68. doi: [10.1016/j.neuroscience.2013.02.063](https://doi.org/10.1016/j.neuroscience.2013.02.063). [PubMed: [23500094](https://pubmed.ncbi.nlm.nih.gov/23500094/)].
- Jolitha AB, Subramanyam MV, Asha Devi S. Modification by vitamin E and exercise of oxidative stress in regions of aging rat brain: studies on superoxide dismutase isoenzymes and protein oxidation status. *Exp Gerontol*. 2006;**41**(8):753-63. doi: [10.1016/j.exger.2006.04.007](https://doi.org/10.1016/j.exger.2006.04.007). [PubMed: [16843630](https://pubmed.ncbi.nlm.nih.gov/16843630/)].
- Guo M, Lin V, Davis W, Huang T, Carranza A, Sprague S, et al. Preischemic induction of TNF-alpha by physical exercise reduces blood-brain barrier dysfunction in stroke. *J Cereb Blood Flow Metab*. 2008;**28**(8):1422-30. doi: [10.1038/jcbfm.2008.29](https://doi.org/10.1038/jcbfm.2008.29). [PubMed: [18414498](https://pubmed.ncbi.nlm.nih.gov/18414498/)].
- Misra DS, Maiti R, Ghosh D. Protection of swimming-induced oxidative stress in some vital organs by the treatment of composite extract of *Withania somnifera*, *Ocimum sanctum* and *Zingiber officinalis* in male rat. *Afr J Tradit Complement Altern Med*. 2009;**6**(4):534-43. [PubMed: [20606774](https://pubmed.ncbi.nlm.nih.gov/20606774/)].
- Navarro A, Bandez MJ, Lopez-Cepero JM, Gomez C, Boveris A. High doses of vitamin E improve mitochondrial dysfunction in rat hippocampus and frontal cortex upon aging. *Am J Physiol Regul Integr Comp Physiol*. 2011;**300**(4):R827-34. doi: [10.1152/ajpregu.00525.2010](https://doi.org/10.1152/ajpregu.00525.2010). [PubMed: [21106913](https://pubmed.ncbi.nlm.nih.gov/21106913/)].



24. Navarro A, Gomez C, Sanchez-Pino MJ, Gonzalez H, Bande J, Boveris AD, et al. Vitamin E at high doses improves survival, neurological performance, and brain mitochondrial function in aging male mice. *Am J Physiol Regul Integr Comp Physiol*. 2005;289(5):R1392-9. doi: [10.1152/ajpregu.00834.2004](https://doi.org/10.1152/ajpregu.00834.2004). [PubMed: 16020519].
25. Nedzvetsky VS, Tuzcu M, Yasar A, Tikhomirov AA, Baydas G. Effects of vitamin E against aluminum neurotoxicity in rats. *Biochemistry (Mosc)*. 2006;71(3):239-44. [PubMed: 16545059].
26. Li Y, Liu L, Barger SW, Mrak RE, Griffin WS. Vitamin E suppression of microglial activation is neuroprotective. *J Neurosci Res*. 2001;66(2):163-70. doi: [10.1002/jnr.1208](https://doi.org/10.1002/jnr.1208). [PubMed: 11592111].
27. Glauert HP. Vitamin E and NF-kappaB activation: a review. *Vitam Horm*. 2007;76:135-53. doi: [10.1016/S0083-6729\(07\)76006-5](https://doi.org/10.1016/S0083-6729(07)76006-5). [PubMed: 17628174].
28. Wu A, Ying Z, Gomez-Pinilla F. The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. *Eur J Neurosci*. 2004;19(7):1699-707. doi: [10.1111/j.1460-9568.2004.03246.x](https://doi.org/10.1111/j.1460-9568.2004.03246.x). [PubMed: 15078544].
29. Cruzat VF, Rogero MM, Borges MC, Tirapegui J. Current aspects about oxidative stress, physical exercise and supplementation. *Rev Bras Med Esporte*. 2007;13:304-10.
30. Ricciarelli R, Argellati F, Pronzato MA, Domenicotti C. Vitamin E and neurodegenerative diseases. *Mol Aspects Med*. 2007;28(5-6):591-606. doi: [10.1016/j.mam.2007.01.004](https://doi.org/10.1016/j.mam.2007.01.004). [PubMed: 17306357].
31. Miller E3, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guller E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med*. 2005;142(1):37-46. [PubMed: 15537682].
32. Van Zutphen LF, Baumans V, Beynen AC. *Principles of laboratory animal science*. Amsterdam. The Netherlands: Elsevier Science Publisher; 2005. p. 373-81.
33. TaheriChadorneshin H, Cheragh-Birjandi S, Ramezani S, Abtahi-Eivary SH. Comparing sprint and endurance training on anxiety, depression and its relation with brain-derived neurotrophic factor in rats. *Behav Brain Res*. 2017;329:1-5. doi: [10.1016/j.bbr.2017.04.034](https://doi.org/10.1016/j.bbr.2017.04.034). [PubMed: 28445707].
34. Malafa MP, Fokum FD, Mowlavi A, Abusief M, King M. Vitamin E inhibits melanoma growth in mice. *Surgery*. 2002;131(1):85-91. [PubMed: 11812968].
35. Carrier JC, Aghdassi E, Jeejeebhoy K, Allard JP. Exacerbation of dextran sulfate sodium-induced colitis by dietary iron supplementation: role of NF-kappaB. *Int J Colorectal Dis*. 2006;21(4):381-7. doi: [10.1007/s00384-005-0011-7](https://doi.org/10.1007/s00384-005-0011-7). [PubMed: 16133010].
36. Naert G, Maurice T, Tapia-Arancibia L, Givalois L. Neuroactive steroids modulate HPA axis activity and cerebral brain-derived neurotrophic factor (BDNF) protein levels in adult male rats. *Psychoneuroendocrinology*. 2007;32(8-10):1062-78. doi: [10.1016/j.psyneuen.2007.09.002](https://doi.org/10.1016/j.psyneuen.2007.09.002). [PubMed: 17928160].
37. Bejot Y, Mossiat C, Giroud M, Prigent-Tessier A, Marie C. Circulating and brain BDNF levels in stroke rats. Relevance to clinical studies. *PLoS One*. 2011;6(12). e29405. doi: [10.1371/journal.pone.0029405](https://doi.org/10.1371/journal.pone.0029405). [PubMed: 22195050].
38. Wu SY, Wang TF, Yu L, Jen CJ, Chuang JI, Wu FS, et al. Running exercise protects the substantia nigra dopaminergic neurons against inflammation-induced degeneration via the activation of BDNF signaling pathway. *Brain Behav Immun*. 2011;25(1):135-46. doi: [10.1016/j.bbi.2010.09.006](https://doi.org/10.1016/j.bbi.2010.09.006). [PubMed: 20851176].
39. Albeck DS, Sano K, Prewitt GE, Dalton L. Mild forced treadmill exercise enhances spatial learning in the aged rat. *Behav Brain Res*. 2006;168(2):345-8. doi: [10.1016/j.bbr.2005.11.008](https://doi.org/10.1016/j.bbr.2005.11.008). [PubMed: 16388860].
40. Rowlands MA, Holly JM, Gunnell D, Donovan J, Lane JA, Hamdy F, et al. Circulating insulin-like growth factors and IGF-binding proteins in PSA-detected prostate cancer: the large case-control study ProtecT. *Cancer Res*. 2012;72(2):503-15. doi: [10.1158/0008-5472.CAN-11-1601](https://doi.org/10.1158/0008-5472.CAN-11-1601). [PubMed: 22106399].
41. Ding YH, Young CN, Luan X, Li J, Rafols JA, Clark JC, et al. Exercise preconditioning ameliorates inflammatory injury in ischemic rats during reperfusion. *Acta Neuropathol*. 2005;109(3):237-46. doi: [10.1007/s00401-004-0943-y](https://doi.org/10.1007/s00401-004-0943-y). [PubMed: 15616790].
42. Lira FS, Yamashita AS, Rosa JC, Tavares FL, Caperuto E, Carnevali LJ, et al. Hypothalamic inflammation is reversed by endurance training in anorectic-cachectic rats. *Nutr Metab (Lond)*. 2011;8(1):60. doi: [10.1186/1743-7075-8-60](https://doi.org/10.1186/1743-7075-8-60). [PubMed: 21861927].
43. Martin A, Janigian D, Shukitt-Hale B, Prior RL, Joseph JA. Effect of vitamin E intake on levels of vitamins E and C in the central nervous system and peripheral tissues: implications for health recommendations. *Brain Res*. 1999;845(1):50-9. [PubMed: 10529443].
44. Ibrahim WH, Bhagavan HN, Chopra RK, Chow CK. Dietary coenzyme Q10 and vitamin E alter the status of these compounds in rat tissues and mitochondria. *J Nutr*. 2000;130(9):2343-8. [PubMed: 10958833].
45. Asha Devi S, Prathima S, Subramanyam MV. Dietary vitamin E and physical exercise: II. Antioxidant status and lipofuscin-like substances in aging rat heart. *Exp Gerontol*. 2003;38(3):291-7. [PubMed: 12581793].
46. Vatassery GT, Quach HT, Smith WE, Santacruz KS, Roy S. Apolipoprotein E deficiency leads to altered brain uptake of alpha-tocopherol injected into lateral cerebral ventricles. *Biochim Biophys Acta*. 2007;1772(7):797-803. doi: [10.1016/j.bbadis.2007.04.006](https://doi.org/10.1016/j.bbadis.2007.04.006). [PubMed: 17560088].
47. Mardones P, Strobel P, Miranda S, Leighton F, Quinones V, Amigo L, et al. Alpha-tocopherol metabolism is abnormal in scavenger receptor class B type I (SR-BI)-deficient mice. *J Nutr*. 2002;132(3):443-9. [PubMed: 11880569].
48. Liu J, Yeo HC, Overvik-Douki E, Hagen T, Doniger SJ, Chyu DW, et al. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *J Appl Physiol (1985)*. 2000;89(1):21-8. doi: [10.1152/jappl.2000.89.1.21](https://doi.org/10.1152/jappl.2000.89.1.21). [PubMed: 10904031].
49. Ristow M, Zarse K, Oberbach A, Klötting N, Birringer M, Kiehnopf M, et al. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A*. 2009;106(21):8665-70. doi: [10.1073/pnas.0903485106](https://doi.org/10.1073/pnas.0903485106). [PubMed: 19433800].
50. Strobel NA, Peake JM, Matsumoto A, Marsh SA, Coombes JS, Wadley GD. Antioxidant supplementation reduces skeletal muscle mitochondrial biogenesis. *Med Sci Sports Exerc*. 2011;43(6):1017-24. doi: [10.1249/MSS.0b013e318203afa3](https://doi.org/10.1249/MSS.0b013e318203afa3). [PubMed: 21085043].