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Original Article

Preconditioning with Endurance and Cognitive-Endurance Training Enhances Brain-Derived Neurotrophic Factor in Aged Rats with Dementia

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A B S T R A C T

Introduction: Sports training has been shown to have a positive impact on conditions such as dementia, but its exact mechanisms remain unclear. The present study aimed to determine the effects of pre-training with endurance and cognitive endurance training on brain-derived neurotrophic factor levels in aged rats after dementia.

Materials and Methods: Male Wistar rats were randomly divided into 4 groups of 10. These groups included the healthy control group, the dementia group, the dementia group with endurance training, and the dementia group with cognitive-endurance training. Trihexyphenidyl was used to create a mouse model of dementia. Swimming and the Morris water maze were considered for endurance and cognitive endurance training, respectively. After the training period, the expression level of brain-derived neurotrophic factor was measured using the Real-Time polymerase chain reaction.

Results: Brain-derived neurotrophic factor expression is significantly decreased in rats with dementia compared to control rats. Exercise had a positive effect on brain-derived neurotrophic factor expression ($p<0.05$). Cognitive- endurance exercise was found to be more effective than endurance exercise.

Conclusion: Endurance and cognitive-endurance exercises can be considered suitable strategies for preventing dementia in the elderly and also for reducing the complications of dementia in affected people.

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Introduction

Age-related atrophy in the frontal, parietal, and temporal regions of the human brain occurs during the third decade of life [1]. Advancing age is associated with alterations in executive functioning, manifesting as challenges in performing instrumental activities of daily living, diminished processing speed, prolonged reaction times, and impaired inhibitory control [2]. Language comprehension, especially of intricate texts, relies on working memory, which declines with normal aging. This decline is a characteristic feature of mild cognitive impairment (MCI) and Alzheimer's disease, where brain atrophy, particularly affecting the hippocampus, has a more significant impact [3, 4]. In fact, brain atrophy occurs in crucial regions before detectable cognitive changes are detected. Brain regions susceptible to atrophy from aging and disease have demonstrated alterations in both structure and function following moderate aerobic exercise. This indicates that engaging in physical exercise can enhance brain health and improve cognitive function [5, 6].

Typical aging is accompanied by modifications in both brain structure and function, as well as associated cognitive changes. While cognitive decline is a recognized aspect of normal aging, some alterations may be indicative of neurodegenerative conditions, including Alzheimer's disease and other types of dementia. The number of people aged 65 and older in the US is predicted to rise from 43 million to 92 million by 2060 [7]. In the United States, the prevalence of dementia among individuals aged over 71 years is estimated at approximately 14%, with Alzheimer's disease accounting for around 10% of these cases. Cognitive impairment not classified as dementia, including MCI, is estimated to affect about 22% of this population, with 12% of those individuals progressing to dementia each year [8]. The majority of research on the mechanisms by which physical exercise influences cognitive performance has focused on aerobic exercise. However, resistance training may affect cognitive function through distinct pathways. Physical exercise has been shown to be especially beneficial for apolipoprotein E4 carriers, who are at increased risk of developing dementia [9].

Neurotrophins, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor, promote plasticity and enhance neurovasculature in certain brain areas, such as the hippocampus [10]. These physiological changes may exert beneficial effects on cognitive functioning in the aging brain [11]. BDNF, a crucial factor in controlling the development, maintenance, and viability of neurons in the mature brain, is elevated in the blood of adults who engage in either brief or prolonged aerobic activity. Subjects who underwent 3 months of endurance training showed a 4-fold increase in resting BDNF levels [10, 12]. In some clinical trials, aerobic exercise appears to be associated with increased levels of BDNF. The majority of these trials have been conducted in young adults, and there is no information on the role of exercise training in older adults. Estimating *BDNF* concentrations within the human brain remains challenging; however, BDNF readily

traverses the blood–brain barrier. Although serum BDNF levels may reflect central concentrations, existing findings are inconsistent, warranting further investigation [13-16].

Physical exercise is essential for preserving physical functioning and physiological health, and it plays a vital role in sustaining brain health and cognitive abilities in older adults. The present study examined the impact of endurance training and combined cognitive-endurance training on dementia by assessing BDNF levels.

Materials and Methods

Preparation of animals

A total of 40 adult male, healthy Wistar rats, weighing 250 grams and of the same age, were obtained from the Pasteur Institute-Iran. The animals were relocated to the college's animal facility, where they were exposed to a temperature range of 20 to 22 °C, a humidity range of 56 to 60%, and a light cycle of 12 hours of light followed by 12 hours of darkness. Water and rat food were available to all animals without any restrictions. Throughout the research, the animals had free access to the water. The animals underwent a one-week adaptation period to minimize the effects of environmental factors, such as displacement, temperature, light, and humidity, on the test results. Over the course of a week, the animals were handled daily to minimize stress from handling and working with them during the experiment.

Mice models of dementia

After one week of acclimation to the new environment, the mice were randomly divided into four groups of 10. These groups included the healthy control group, the dementia group, the endurance group, and the endurance-cognitive group. Temporarily weakening the memory of mice and inducing dementia was done using the drug trihexyphenidyl. The drug was administered intravenously to the mice for 5 days at a dose of 250 mg/kg body weight.

Endurance training

The endurance training group swam in a pool for rodents for 3 weeks, 5 days a week, as part of their training protocol. A special pool measuring 50×50×100 cm was used by the endurance-training group rats to swim once a day, five days a week. The main swimming training program started at 30 minutes and increased by 5 minutes per day to 60 minutes in the second week. Until the end of the third week, the 60-minute time was set. Adjusting the strength and speed of the water during swimming was the method used to perform training overload, and it was consistent throughout the training adaptation week. During the training weeks, the speed and power of the water flow increased from 7 to 15 liters per minute by keeping the time constant at 60 minutes [17].

Cognitive-endurance training

The cognitive-endurance training group followed a procedure in which each rat underwent Morris water maze training sessions over 3 weeks. The training was conducted three days per week, with four sessions per day and a ten-minute break between each session. The Morris water maze device consisted of a black cylindrical basin with a diameter of 136 cm and a height of 60 cm, filled with water to a height of 25 cm. The water temperature had been set to 20 °C. A small platform made of dark-painted metal with a

diameter of 10 cm was located 1 cm below the water surface in the center of the southwest quadrant of the circle. The experiments were conducted in a relatively dark room, with visible signs installed on its four sides, and the animal could use these signs to locate the hidden platform. The mouse was randomly released from one of the quadrants of the pond, and the experimenter recorded the time it took to find the platform. Each experiment began with the animal released into the water from a starting point (north, south, east, or west) with its face facing the cylinder wall. In every turn, one of the four starting points was utilized. The experiment is conducted when the mouse steps onto the platform or 90 seconds have passed. Then, the animal was given 30 seconds, after which the next experiment began. The rats that could not locate the platform were moved to it by the experimenter and allowed to stay there for 30 seconds. After the fourth experiment was completed, the mice were removed from the pond [18].

Expression level of *BDNF*

The reverse transcription polymerase chain reaction (RT-PCR) method was used to quantify *BDNF* gene expression. RNA was extracted from the brain tissue of rats using the Trizol kit and following the manufacturer's instructions. The cDNA synthesis was performed using a commercial kit, random hexamer, and Oligo (dT) primers, after evaluating RNA quantitatively and qualitatively by spectrophotometry and agarose gel electrophoresis. To perform the RT-PCR, 5 µl of SYBR green master mix, 0.5 µl of each forward and reverse primer (10 µM), and 1 µl of cDNA sample were combined to a final volume of 10 µl with double-distilled water. The forward and reverse primers designed for *BDNF* gene amplification included: 5'-ATTTAATCCGGCGAATTCTC-3' and 5'-AATAGGCACTGGGCAACCAGGGGAT-3', respectively. The temperature profile started with an initial denaturation at 95°C for 5 minutes. Then, 35 cycles were performed, including denaturation at 95°C for 15 seconds, annealing at 60°C for 15 seconds, and extension at 72°C for 20 seconds. *β-actin* housekeeping gene was used to normalize the data.

Statistical analysis

Using the data obtained from gene expression analysis, the amount of fold change was calculated through the formula $2^{-\Delta\Delta Ct}$. To examine significant differences between groups, the normality of the data distribution was assessed using the Shapiro-Wilk test to ensure the validity of the parametric assumptions. Then, Tukey's test and GraphPad Prism software were employed. At all steps, $p < 0.05$ was considered significant.

Results

Quantitative study of *BDNF* expression

The relative standard method was used to calculate the amount of mRNA. This method compares the expression of the target gene to that of an internal control gene, typically a housekeeping gene. In this research, β-actin was used as a reference gene. Analysis of the melting curves for the *BDNF* and *β-Actin* genes revealed a single peak at the Tm, indicating the specificity of the PCR reaction for both genes (Figure 1). The electrophoresis pattern also confirmed this

issue. The results of *BDNF* gene expression, expressed as fold change, are shown in Figure 2 after normalization to the reference gene. It is evident that the expression of this gene decreased significantly in the sham (dementia) group compared to the control group. Additionally, both endurance training and endurance-cognitive training enhance *BDNF* gene expression, resulting in levels that closely resemble those of the control samples. Notably, the levels of *BDNF* expression in the endurance training and cognitive-endurance training groups did not differ significantly from those in the control group.

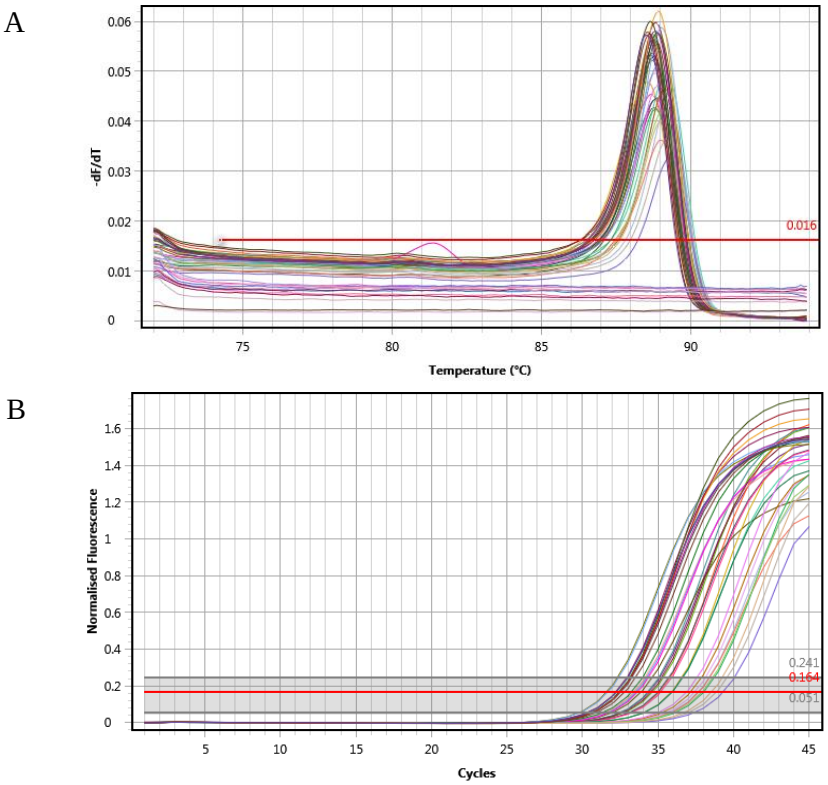

Fig. 1. Melting (A) and amplification (B) curves of the *BDNF* gene

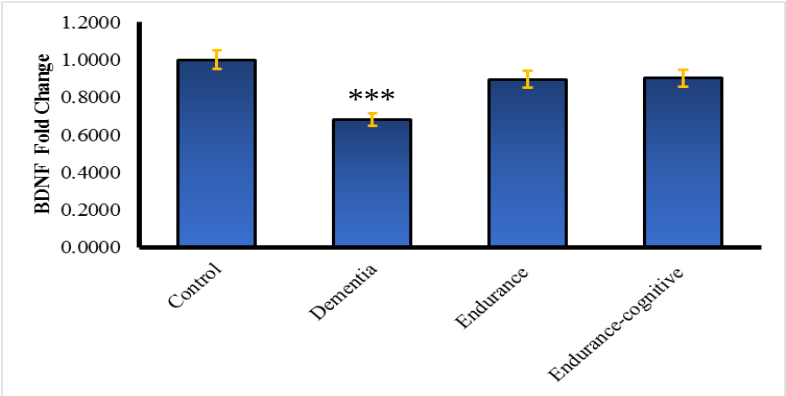

Fig. 2. Comparison of the expression level of *BDNF* in the brain tissue of the studied mice (***p<0.001)

Discussion

The expression of *BDNF* in dementia model mice was evaluated by assessing the effects of two types of endurance training and endurance-cognitive training. Compared to control rats, sham (dementia) rats displayed a significant decrease in *BDNF* gene expression. Both endurance and endurance-cognitive training resulted in an increase in *BDNF* gene expression. The gene expression levels in the rats of the training group were very similar to those of the control samples. The expression level of *BDNF* in the endurance training and endurance-cognitive training groups did not differ significantly from the control group. There was no significant distinction in the changes in *BDNF* gene expression between endurance training and endurance-cognitive training.

Physical exercise has the potential to prevent degenerative brain changes associated with aging and neurological diseases by modulating abnormal protein deposition, increasing neurotrophic factors, improving cerebral blood flow, and reducing systemic inflammation. Animal research has shown that exercise promotes compelling neuroplasticity in the brain. Early research on rodents revealed that accessing exercise equipment (treadmills) helped neurons grow and strengthened connections within systems involved in learning and memory. Increased levels of neurotrophic factors in the brain are associated with exercise in mice. According to Cotman and Berchtold, rats that exercised had higher *BDNF* gene expression in the hippocampus than those that did not. BDNF levels in the hippocampus were highly correlated with the distance run per night [19]. Mice who engaged in voluntary treadmill exercise demonstrated improved learning and memory, as evidenced by better performance in the water maze. The decrease in β-amyloid deposition in exercising rats was linked to these behavioral changes. Exercise also increases blood flow to the brain, thereby facilitating nerve growth and neural function in the hippocampus and promoting the growth and protection of neural structures [20].

Acute resistance training was studied by Hosseini et al. to determine its effects on plasma BDNF levels in rats. In this study, 25 female Wistar rats were randomly divided into two groups: a control group and a resistance training group, in which carrying weights while climbing a ladder was considered resistance training. The findings of this research showed that changes in plasma BDNF levels following a resistance training session at the time points immediately, 24, and 72 hours were greater than in the control group [21]. After endurance and cognitive endurance training, the present study showed that *BDNF* expression increased in the brains of rats with dementia.

Yaghoubi and Elhami studied 6 weeks of high-intensity circuit resistance training to determine its effect on BDNF levels in men who are not active. 24 hours before the start of training and 48 hours after the last training session, blood was collected from the subjects,

and changes in BDNF levels were measured by the enzyme-linked immunosorbent assay. Six weeks of resistance training resulted in a significant increase in the subjects' BDNF levels. The resistance training group had a significantly higher plasma BDNF level than the control group [22]. According to the results of this study, it can be concluded that resistance training likely plays a beneficial role in synaptic plasticity in inactive individuals by increasing BDNF levels. The aforementioned research was conducted on human subjects, and plasma BDNF levels were measured. The present study involved experiments on model mice and the evaluation of *BDNF* expression levels in the brain. Both studies demonstrated that exercise training is effective in boosting BDNF levels. Fazelzadeh et al. investigated BDNF serum levels in active men acutely. Both aerobic and anaerobic acute exercise resulted in a significant increase in BDNF [23]. These results also align with current research on the role of exercise in increasing BDNF. Valipour and Motamedi looked into how a circuit training session affects serum BDNF and insulin-like growth factor (IGF)-1 levels in older individuals. Ten elderly men and women were the subjects of this study. According to the findings, BDNF serum concentration increased significantly, while IGF-1 decreased significantly. Also, the difference between men and women in BDNF and IGF-1 serum concentrations was not significant; Although the changes of BDNF and IGF-1 were lower in men than in women [24].

Etnier et al. examined the effects of acute exercise on memory and BDNF. The study aimed to explore the dose-response relationship among exercise intensity, memory performance, and BDNF levels. Young adults participated in three exercise sessions at varying intensities relative to their ventilatory threshold, each lasting approximately 30 minutes. Following each session, participants completed the ray auditory verbal learning test to evaluate short-term memory, long-term memory acquisition, and recall. A recognition component of the ray auditory verbal learning test was administered 24 hours later to further assess long-term memory. Blood samples were collected prior to exercise, immediately post-exercise, and after the 30-minute recall test. Findings indicated that long-term memory performance varied with exercise intensity, with the greatest improvements observed after maximal-intensity exercise. BDNF levels significantly increased in response to exercise, but no differences were found across exercise intensities, nor was there a significant correlation between BDNF changes and memory measures [25].

Inoue et al. examined the impact of high- and moderate-intensity exercise on BDNF levels and cognitive performance in obese adults. The study aimed to determine whether varying intensities of aerobic exercise influence abdominal fat, BDNF isoforms, and executive functioning. Twenty obese men were randomly assigned to either a moderate-intensity continuous training or a high-intensity interval training group, each exercising three times per week for six weeks,

with matched energy expenditure per session (approximately 300 kcal). Both groups demonstrated a significant increase in BDNF levels immediately following acute exercise, both before and after the intervention. Additionally, executive function improved following the training. Overall, exercise-induced enhancements in BDNF concentration and executive performance were comparable between obese men in the high-intensity interval training and moderate-intensity continuous training groups [26].

Exercise, which aims to address modifiable risk factors and stimulate neuroprotective mechanisms, offers a non-drug strategy for treating age-related cognitive impairment in elderly individuals. Exercise of higher intensity has been found to be associated with lower incident risks of developing cognitive impairment and dementia. Considerable evidence indicates that regular physical activity can induce metabolic, structural, and functional changes in the brain, thereby protecting against age-related loss of cognitive function. However, further studies are necessary to establish the most effective components of exercise programs. Existing studies recommend systematic, prolonged, and multidimensional exercise programs to boost both cognitive function and overall performance among older adults. This work demonstrated that endurance and cognitive endurance training may prevent issues arising from dementia, as indicated by changes in *BDNF* expression. Thus, exercise training must be recognized as a treatment and a preventive measure against dementia related to aging.

Conclusions

This investigation demonstrates that both endurance and cognitive endurance training effectively increase *BDNF* expression in dementia model rats, thereby reversing the decline observed in untreated dementia conditions. These findings support the role of physical exercise as a viable, non-pharmacological strategy to prevent or mitigate dementia-related neurodegeneration by enhancing neuroplasticity and cognitive function. The use of real-time PCR to measure *BDNF* expression provides a reliable and sensitive method to assess the molecular impact of exercise interventions. Future research should explore additional biomarkers and longer-term functional outcomes to elucidate further the protective mechanisms of exercise on brain health in aging populations.

Ethical Considerations

The Ethics Committee of Gerash University of Medical Sciences and Health Services, Gerash, Iran, approved this study under the Ethics code (IR.GERUMS.REC.1403.006).

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Conflict of Interest

The authors declared no conflict of interest.

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Authors' Contribution

A.D: Study concept and design; M.TK: Performing experiments; M.M: Sample collection, NA. S: Analysis; M.TK: Interpretation of data; A.D: Drafting of the manuscript.

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