The effect of eight weeks of endurance training on the hippocampal concentration of tumor necrosis factor alpha in Female rats with Alzheimer's disease

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Abstract

Introduction:
Alzheimer's is a neuro-degenerative disease associated with a decline in memory abilities and brain changes that ultimately lead to dementia. The present study was conducted to investigate the effect of eight weeks of endurance training on the hippocampal concentration of tumor necrosis factor alpha (TNFα) in female rats with Alzheimer's disease.

Materials and Methods:
A total of 30 female Sprague Dawley rats were randomly divided into a control group, a sham group and an endurance training group. Alzheimer's was induced to the rats through Methyltin Chloride. The endurance training group exercised on the treadmill for eight weeks. The hippocampal concentration of TNFα was then measured in all the groups. The data obtained were analyzed using the two-way ANOVA and Tukey’s post-hoc test.

Results:
The hippocampal concentration of TNFα was significantly lower in the exercise group compared to the sham group. Methyltin chloride poisoning increased this concentration and endurance training reduced it in the rats (P≤0.05).

Conclusion:
Endurance training reduces the hippocampal concentration of TNFα in female rats with Alzheimer's disease.

Keywords: Alzheimer, Tumor Necrosis Factor Alpha, Memory, Hippocampus

Introduction
Alzheimer's disease is a neurodegenerative disease of old age that is diagnosed with dementia and reduced neuronal cells in the brain, especially in the brain of older adults (1). The most important cognitive finding of Alzheimer's disease is the deposition of two beta-amyloid peptide tau stranded proteins in the brain. Beta-amyloid peptide can be found in old amyloid plaques in extracellular space in Alzheimer's patients. Amyloid plaques, in addition to beta-amyloid, contain other proteins such as apolipoprotein E that are encoded by a gene predisposing to Alzheimer's disease (APOE) (2). Special pathology of the disease includes a severe
brain atrophy along with the thinning of the "gray matter", the enlargement of the ventricles indicating neuronal lysis., Microscopic extracellular amyloid plaques contain dense amyloid protein, dense protein and cerebrovascular amyloid (amyloid protein surrounding blood vessels.) (3). A group of amyloid degrading enzymes such as NEP (Neprilysin) that exists in a healthy brain help maintain the levels of β-amyloids in low physiological concentrations. Expression and activity of these enzymes decrease under some pathological conditions or aging, contributing beginning of Alzheimer's disease. NEP overexpression may be an appropriate strategy to prevent the accumulation of β-amyloid plaques and the prevention of disease progression (4). Inflammation plays a major role in the development and progression of Alzheimer's. The accumulation of Aβ (beta amyloid) in Alzheimer's disease is associated with an inflammatory response induced by the activation of microglia and astrocytes aggregation which induces the expression of pro-inflammatory cytokines. By stimulating the synthesis of Aβ and amyloid formation, cytokines create a vicious circle in the inflammatory recycling system, and increase the excessive production of reactive oxygen species and oxidative stress, which cause brain damage (5). Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine that plays an important role in the response to injury in the central nervous system. Cytokines are defined as soluble hormone-like proteins. However, compared to hormones that are synthesized by special endocrine tissues, cytokines are secreted by a variety of cells including immune cells, endothelial cells and adipocytes. In addition, their synthesis is activated by a large set of stimulants including free radicals, tissues layers and infectious agents (6). Production of proinflammatory cytokines as one of the inflammatory pathways in the central nervous system is responsible for much of the brain damage (7).

Intense exercise increases the production of free radicals and inflammatory responses in athletes. Strengthening and improving the athlete's immune system can reduce the harmful effects of maximum activity. Intense exercise is associated with immunoassays changes including the release of inflammatory mediators, activities with different types of white blood cells, the activity of acute phase proteins and the increased activity of inflammatory cytokines. Some sports researchers believe that long-term high-intensity exercise can increase the production of free radicals which damage cells and accelerate the aging process (8). Trimethyltin chloride is a potential neurotoxic substance associated with the selective death of neurons in the limbic system of humans and animals, particularly in the hippocampus (9). Trimethyltin causes the necrosis of neurons in the hippocampus and piriform cortex and increases the levels of necrosis factor alpha in the hippocampus. The first sub-cellular changes include the formation of dense collections of multi-core vesicles from tubules and membrane vacuoles in the cytoplasm of proximal and Perry Carrion dendrites (10). Studying the effects of exercise adaptation, Adamopoulos et al. (2001) reported the effects of 12 weeks of exercise training on peripheral inflammatory markers in patients with chronic heart failure. That training program included a thirty-minute daily workout on the ergometer bike with 70 to 80 percent of maximum heart rate and 5 days per week was associated with a significant decrease in TNF-α (11). Based on other studies on the effects of different sports on the level of TNF-α in different diseases and given the fact that all the studies have been conducted on humans; the need for the design of this study is identified. With regard to the aforesaid, the present study seeks to answer this question whether endurance training can
affect the TNF-α hippocampal level in female rats with Alzheimer’s disease.

Materials and Methods
Given the nature of this issue and our objectives, the present study is experimental. Thirty eight-week rats with the mean weight of 10±250 g were purchased from Shiraz Pasteur Institute. After being transferred to the laboratory of Fars Science and Research University and being adapted to the new environment and learning how to use the treadmill, they were randomly divided into three groups: (1) control, (2) sham, (3) endurance training (Table 1). During the study, all the rats were housed in groups of four in transparent polycarbonate cages and at a temperature of 20-24 °C, humidity of 45%55% and 12-h light/dark cycle and 10 g per 100 g bw of the rat feed pellets from Shiraz Stem Cell Center of Nurturing and Breeding of Laboratory Animals was given to them according to weekly weighing (Table 2). The rats had free access to water.

To conduct this research, a week after their adaptation with the environment, the process of developing Alzheimer’s disease in rats began by the administration of trimethyltin chloride. To prepare trimethyltin solution (TMT) and injecting it to rats, 80 mg of this substance was dissolved in 10 ml of normal saline solution (1 ml per 1 kg bw). The training program started a week after the induction of Alzheimer’s. The rats in the control group did no exercise and received no treatment. The rats in sham group received a single dose of 8 mg per kg bw of trimethyltin intraperitoneally.

Blood samples were taken from rats 72 hours after the last training session in the eighth week. The rats were not given feed 4 hours before being slaughtered, but they had access to water and were anesthetized by intraperitoneal injection of anesthetic (a combination of ketamine and xylazine). Then, their hippocampus was removed from the rat’s brain. To obtain total protein of the hippocampus, the removed hippocampus was quickly frozen and crystallized. Then the tissue was ground with a pestle to break the cell walls. After this, all the cell content was extracted and phosphate buffered saline was added to make a solvent. This solution was centrifuged for 10 min at 4000 rpm using a 10-channel device (Sigma, Germany). Finally, the total protein in the hippocampus was used for ELISA study.

Exercise time was set in the afternoons from 14:00 to 17:00 p.m.

Endurance training protocol
Endurance training protocol included eight-week incremental running on a treadmill (Powerjog, England), especially made for rats without gradients (slope of zero percent) with the velocity of 15 to 20 meters per minute for a period of 15-30 minutes per session and five sessions per week. The entire training period was divided into three stages of familiarity (one week), overload, maintenance and stabilization of the labor intensity (seven weeks). The rats walked on the treadmill in the familiarity stage for 5 to 10 minutes at the speed of 5-8 meters per minute every day and at overload stage, first15 minutes at the speed of 15 meters per minute and gradually over a period of two weeks, the intensity and duration of activity was increased to reach the final time of 30 minutes and the speed of 20 meters per minute (equivalent to 50 to 55 percent of maximum oxygen consumption) in the stage of preservation or stabilization. In addition, in each training session, 5 minutes was assigned for warming up and 5 minutes for cooling down (12).

Statistical tests
For data analysis, descriptive and inferential statistics (ANOVA and Tukey’s post hoc test) were used. All statistical tests were performed at a significance level P ≤ 0.05.

Results
Kolmogorov-Smirnov test results showed that the distribution of variables is normal.
in research groups. The results of analysis of variance and Tukey’s tests showed that after treatment with trimethyltin in the sham group, the hippocampal level of TNF-α was significantly higher than that in the control group (p=0.0001). The studies also showed that following treatment with endurance training TNF-α levels significantly reduced. Also, the comparison of sham and endurance training groups showed a significant difference of (p≤0.0001). The test showed that the toxicity with trimethyltin significantly increased TNF-α hippocampal concentrations in female rats, while endurance training reduced its amount. The amount of this agent was 51.10±13.14 pg ml in the control group, 127.28±11.18 pg ml in the sham group and 62.91±18.16 pg ml in the training group (Figure 1).

As Table 1 shows, in the control group, rats' body weight increased over time while following treatment by trimethyltin, their weight decreased over time in sham and training groups. (Table 1)

![Graph showing changes in hippocampal TNF-α (female) in the study groups; ** Or ++ statistically significant difference (** compared to control group and + + compared to sham group)](image)

Table 1: Rats' body weight on the first day, first, fourth and eighth weeks (data presented as mean and standard deviation)

<table>
<thead>
<tr>
<th>groups</th>
<th>First day</th>
<th>First week</th>
<th>4th week</th>
<th>8th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>204.9±19.9</td>
<td>208.4±20.8</td>
<td>221.1±21.8</td>
<td>229.2±19.3</td>
</tr>
<tr>
<td>Sham</td>
<td>199±19.5</td>
<td>195.5±19.2</td>
<td>185±20.1</td>
<td>177.3±10.7</td>
</tr>
<tr>
<td>Training</td>
<td>170.5±15.1</td>
<td>167±16.1</td>
<td>147.8±18.5</td>
<td>136.1±18.1</td>
</tr>
</tbody>
</table>

**Discussion**

Endurance training reduces the amount of plasma concentrations of TNF-α and its receptors (13) and interleukin-6 (IL-6) (14). Some researchers have also suggested that those who do more physical activities and are physically fit have lower concentrations of inflammatory markers compared to those who are inactive and sedentary (15). Endurance exercise can also reduce cytokine gene expression in muscle tissues (16) or reduce the daily instances of hypoxia (stimulator of proinflammatory cytokines gene expression) by strengthening the cardiorespiratory system and reducing proinflammatory cytokines from mononuclear cells (17).
The present study shows that endurance training for eight weeks reduced hippocampal concentrations of TNF-α in female rats with Alzheimer's disease compared to those in the sham group, which shows the effect of endurance training on the reduction of TNF-α. Yu et al. showed that doing aerobic exercises had no effect on serum TNF-α levels while it reduced its receptors. They revealed that failure to reduce TNF-α with endurance training can be due to its temporary production and its short half-life, while the reduction in its receptors can reflect the more real performance of TNF-α. Nick Las et al. studied independent and combined effects of exercise (aerobic and resistance) and diet on inflammatory markers and concluded that exercise had no effect on the CRP, IL-6 and TNF-α markers while weight loss decreased the mentioned markers. Meanwhile, Yanakolya et al. concluded that 12 weeks of endurance training improved insulin sensitivity in overweight and obese girls, while it causes no significant changes in body weight, body fat and inflammatory markers such as TNF-α. Mogharnasi et al. found that regular and long-term endurance training (5585% of maximum oxygen consumption) by reducing the levels of TNF-α and inflammation plays an effective role in prevention, control and reduction of atherosclerosis. Research of Ravasy et al. showed that endurance training significantly decreased serum levels of TNF-α and IL-6 in overweight men. The results of the present study are similar to those of some previous studies and its contradiction with the previous studies may be due to differences in the type, intensity and duration of the exercises or not following a diet, fewer training sessions per week, mental stress or even racial factors in the subjects. Due to changes in neuronal density in the hippocampus after treatment with trimethyltin, it is suggested that the improvement of neuronal density in the hippocampus following the administration of different doses of lithium chloride along with endurance training be studied. Another result of this study, following the administration of trimethyltin and also after having endurance training, is weight loss which has also been reported in other studies. For example, epileptic seizures, weight loss and behavioral changes (hyperactivity, tail wagging, making sound, hypersensitivity and aggressiveness) are seen in lab animals following the administration of trimethyltin. Some indications of cognitive impairment such as memory loss and impaired learning that is associated with hippocampal damage have also been noticed.

Conclusion
With regard to our findings, it can be concluded that endurance exercise program reduces the hippocampal TNF-α concentrations in the rats with Alzheimer's.

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Conflict of interest
The authors have announced no conflict of interest with regard to the authorship and / or publication of this article.

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