The effect of running on positive and negative slopes on TNF-α and INF- γ gene expression in the muscle tissue of rats with Alzheimer's disease

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Abstract

Introduction: Alzheimer's disease (AD) is a disease associated with disorders of the nervous system. Inflammation in the central nervous system can play a major role in the formation of AD. This study aimed to investigate the effect of AD induction as well as running on positive and negative slopes on TNF-α and INF-γ gene expression in the muscle tissue of rats with AD.
Materials and methods: In this experimental study 15 rats were injected with 8 mg/kg Trimethyltin chloride (TMT) intra- peritoneally. After being assured of AD rats were divided in three groups of 5 rats, including: 1) control, 2) running in positive slope, and 3) running in negative slope. To investigate the effects of AD induction on the TNF-α and INF-γ gene expression levels, 5 rats were assigned to the healthy control group. During eight weeks the positive running group (at a speed of 16 m/min in positive upward slope) and negative running group (at a speed of 16 m/min in negative downhill slope) ran on the treadmill for five sessions per week and 60 minutes per session. The Shapiro- Wilk test was used for investigate the normal distribution of data and one-way ANOVA with LSD *post- hoc* tests were used to analyze the data ($P<0.05$).
Results: AD induction by TMT significantly increased TNF-α ($P=0.004$) and INF-γ ($P=0.04$), nevertheless running on positive and negative slope significantly decreased TNF-α ($P=0.001$) also running on negative slope significantly decreased INF-γ ($P=0.04$).
Conclusion: Running on a positive and negative slope seems to improve TNF-α gene expression in rats with AD.

Keywords: Running, Alzheimer's disease, TNF-α, INF- γ

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that is diagnosed by a lack of memory and perception, and this impairment of memory is implicated in daily functioning and life of people. AD is now increasing at an alarming rate in industrialized countries (1). The process of injury in AD begins in the hippocampus part of the brain. The hippocampus is a part of the brain responsible for storing information about short-term memory and plays an important role in learning. Inflammation in the brain is a defensive response to microbial contamination and plays a role in the development of pathological behaviors and tissue damage. These pathological changes

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are associated with many neuronal disorders such as neurodegenerative impairment (2). Several gene products involved in the primary immune response are important in spreading inflammatory responses such as brain inflammation. Studies have shown that inflammation in the central nervous system can play an important role in the formation of AD (3). Mild chronic inflammation can be an essential issue in the pathophysiology of several chronic diseases, especially AD (4). Mild chronic inflammation is associated with higher than normal levels of several cytokines such as interleukin- 6 (IL-6) and tumor necrosis factor- α (TNF-α) (5). When Alzheimer's causing factors such as beta amyloid accumulation and accumulation of Tau proteins are hyperphosphorylated, they stimulate glial cells and astrocytes, which this stimulation produce IL- 6, IL- 1β and TNF-α; on the other hand, pro- inflammatory factors and cytokines cause destruction of neurons (6); which its result is cognitive impairment (6). It has been reported that TNF-α can induces a wide range of cellular responses. TNF-α is present in a small amount in the normal brain. Nevertheless, pathological stimuli (like infection, ischemia and injury) can significantly raise the TNF-α expression. High levels of TNF-α expression are involved in the pathogenesis of some human brain disorders like AD and Parkinson's disease (1). Interferon- gamma (INF-γ) is also produced by natural killer cells or activated T cells during immune responses. This protein exhibits anti-proliferative, immune regulation and pro-inflammatory activity and is therefore important in defense mechanisms (3). On the other hand, the decrease in physical activity in patients with disorders of the nervous system inflammation is associated with the occurrence of other chronic diseases, which is associated with a progressive decrease in the motor capacity of these patients (7). Studies have reported an increase in inflammatory factors in skeletal muscle with increasing age and decreased physical activity (8). In addition, numerous factors following neurodegenerative damage to neural axis, such as the hypothalamic pituitary, can lead to decreased motor ability in a variety of pathways. Studied have shown that decreased adrenocorticotropic function in central nervous system disorders is related with decrease in testosterone levels, peripheral insulin resistance, decrease in insulin-like growth factor-1 (IGF-1) and increase in inflammatory factors (9). Over the past decade, regular exercise has been shown to have beneficial effects on brain function. Brain tissue, like other tissues, responds to exercise. In a way, regular exercises have a positive effect on perception and cognition by improving learning and memory, preventing neurological disorders such as anxiety, depression, Parkinson's and AD as well as delaying memory loss due to aging. Studies have also shown that in humans, physical exercise delays the onset of neurological damage (10); however, the results of studies investigating the anti-inflammatory effects of exercise in patients with AD are contradictory. The results of some studies indicate the effect of exercise on reduction (11, 12) and some increase (13, 14) of inflammatory factors. Given the limitations of research on AD disease, researchers use AD animal models for fundamental research. Trimethyltin (TMT) is a specific neurotoxin that increases levels of reactive oxygen species and activation of the pathways of inflammation and apoptosis as well as affects different parts of the central nervous system (15).

Considering the contradictory results of the noted researches and the possible effects of exercise on different slopes on inflammatory factors and lack of research in comparing the effects of running on positive and negative slopes; aim of this study was to investigate the effect of AD induction by TMT on TNF-

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α and INF-γ gene expression in muscle tissue of rats as well as investigate the effects of running on positive and negative slopes on TNF-α and INF-γ gene expression in muscle tissue of rats.

Materials and methods

In this experimental study, 20 male Sprague-Dawley rats were purchased and kept for one week in the laboratory environments with standard temperature, humidity and lighting conditions. Then, on eighth day, 15 rats were injected intra- peritoneally with 8 mg/kg of TMT (2) and 3 days later after confirm of hippocampus degeneration (AD symptoms were observed by a number of behavioral symptoms in rats. These clinical symptoms were included: 1) muscle tremors, 2) elevated body temperature, 3) nausea, 4) seizures, and 5) tail twists). Then, the rats with AD were randomly divided into 3 groups of 5 rats including 1) control, 2) positive slope training, and 3) negative slope training groups. It is noteworthy that in order to investigate the effects of AD induction on TNF-α and INF-γ levels, 5 rats were assigned to the healthy control group. During eight weeks the rats in the positive slope trainings group (16 m/min in positive, upward slope) and negative slope training group (16 m/min in downward slope) performed 60 minutes endurance trainings per session for five sessions per week (16). To perform training, rats initially ran on the treadmill with zero slope for 5 min at a speed of 8 m/min for warm-up; afterwards, the training groups performed training at a speed of 15 cm/s in the first week at +15% and -15% slopes, adding 5 cm/s to the treadmill speed each week. At the end of each training session, rats cooled down again for 5 min at 8 m/min on the treadmill with zero slopes (16). It should be noted that all training sessions performed between 9 am to 12 am as well as the rats in control group were rats with AD but in healthy control group were intact rats (healthy rats) indeed during research period the control and healthy control groups did not performed positive and negative slope trainings. Forty eight hours after the last training session, rats were anesthetized with *ketamine* 10% (50 mg/kg dose) and *xylazine* 2% (10 mg/kg dose) after approximately 5 minutes.

It should be noted that Due to decreased ability of physical activity in patients with disorders of the nervous system inflammation and neurotransmitter impairment (through damage to the nerve axis such as the hypothalamic- pituitary, adrenocorticotropin impairment, growth hormone releasing hormone, growth hormone abnormality, insulin-like growth factor-1 (IGF-1), decreased testosterone, increased insulin resistance); neurodegenerative disorders develop in skeletal muscle of elderly people (7,8) and patients with AD. As a result, this muscle weakness increases the risk of falls (also known as fall syndrome) (9), so in this study, the researcher is looking for a way to reduce inflammatory factors in the muscle tissue to reduce muscle atrophy and prevent it in patients with AD. So the soleus muscle tissue was extracted by specialists and after setting in cryotube was placed in liquid nitrogen and stored at -70 °C for further investigation. The genes expressions were measured by quantitative q-RT PCR method. The sequence of primers used is shown in Table 1. The Shapiro-Wilk was used for

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investigate the normal distribution of data and one-way ANOVA with LSD *post- hoc* tests were used for statistical analysis of data (P<0.05).

Table 1. Sequence of forward-reverse primers of TNF-α, INF- γ and reference genes for Real-time PCR reaction.

Genes	Primer Sequences	Sizes (bp)
β2M	Forward: 5'- CGTGCTTGCCATTCAGAAA-3'	244
	Reverse: 5'-ATATACATCGGTCTCGGTGG-3'	
IFN- γ	Forward: 5'- AGGATGCATTCATGAGCATCGCC-3'	136
	Reverse: 5'- CACCGACTCCTTTTCCGCTTCCT-3'	
TNF-α	Forward: 5'- ATGGGCTCCCTCTCATCAGT-3'	106
	Reverse: 5'- GCTTGGTGGTTTGCTACGACG-3'	

Results

The levels of TNF-α and INF-γ gene expression in the muscle tissue of rats in the four groups of study are presented in Figures 1 and 2, respectively. The results of Shapiro-Wilk test showed that distribution of TNF-α (P=0.80) and INF-γ (P=0.10) gene expression in four groups of study were normal.

The results of one-way ANOVA showed that there were significant differences in TNF-α (F=10.37; P=0.001) and INF-γ (F=4.37; P=0.02) gene expression in the study groups. The results of LSD post hoc test showed that TNF-α (P=0.004) and INF-γ (P=0.04) gene expression levels were significantly higher in the control group than healthy control group, so AD induction by TMT significantly increased TNF-α and INF-γ gene expression (Figure 1 and 2 respectively); TNF-α gene expression levels in the positive (P = 0.001) and negative (P = 0.001) slope training groups were significantly lower than the control group; there were no significant differences in TNF-α gene expression levels between negative and positive slope training groups (P=0.15) (Figure 1); and INF-γ gene expression levels in positive slope training group were significantly lower than control (P = 0.04) and negative slope training (P=0.01) groups (Figure 2).

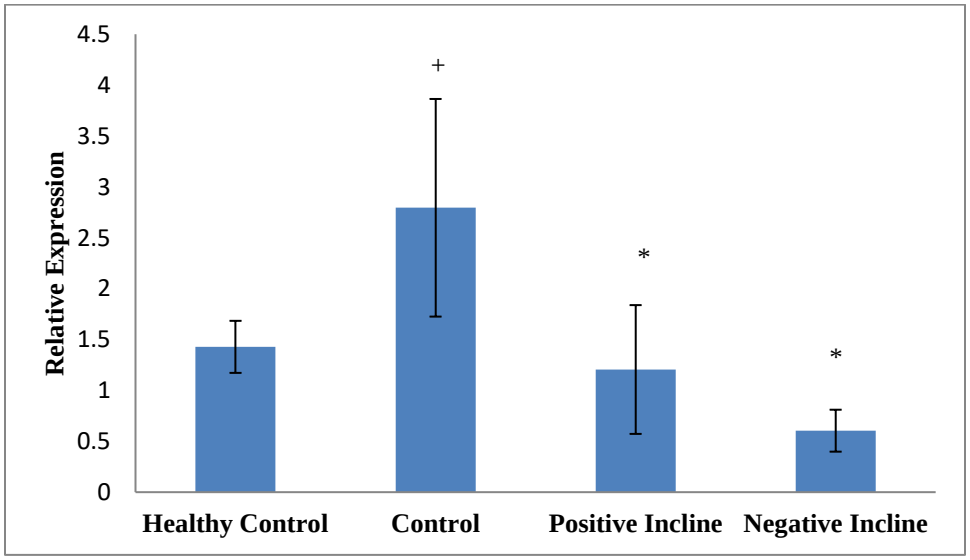


Figure 1. TNF-α gene expression levels in the four research groups.
[+]P<0.01 Significant increase compared to healthy control group.
[*]P<0.001 Significant decrease compared to control group.

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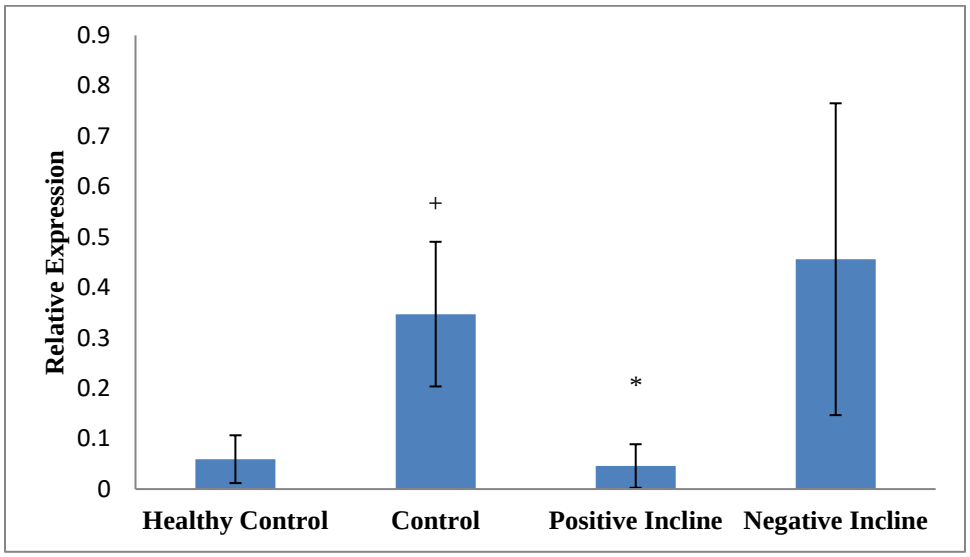


Figure 2. INF- γ gene expression levels in the four research groups.
[+]P<0.05 Significant increase compared to healthy control group
[*]P<0.05 Significant decrease compared to control group

Discussion

Present study aimed to investigate the effect of AD induction by TMT on TNF-α and INF-γ gene expression in muscle tissue of rats as well as investigate the effects of running on positive and negative slopes on TNF-α and INF-γ gene expression in muscle tissue of rats. The results showed that TNF-α and INF-γ gene expression in control group were significantly higher than healthy control group which means AD induction by TMT significantly increased TNF-α and INF-γ gene expression in muscle tissue of rats. Also the results showed that running on positive and negative slope significantly decreased TNF-α gene expression also running on negative slope significantly decreased INF-γ gene expression in rats with AD. Studies have shown that any damage to the motor system from the motor cortex to the motor neuron will be associated with muscle loss and atrophy (17). Recent studies have also shown that chronic inflammation in the muscle mass is associated with increased levels of free radicals, DNA damage, and cell death following aging and neurological diseases (18). However, exercise seems to induce anti-inflammatory effects on muscle by different mechanisms. Broderick *et al.* believed that exercise can decrease CRP levels by increasing nitric oxide synthase levels, enhancing endothelial growth factors, improving metabolism as well as improving the function of glucose transporter within muscle cells, which this factor in turn inhibits inflammatory cytokines (19); also in Dalle *et al.* study it has been shown that aerobic exercises decrease muscle atrophy by increasing anti-inflammatory factors in muscle tissue (such as IL-10) and inhibiting inflammatory factors (such as TNF-α and IL-1β); but resistance exercises prevent the progression of inflammation through activation of Akt/mTOR pathway and inhibition of NFkB (18). Therefore, it seems that exercises in a variety of ways can have anti-inflammatory effects on muscle tissue.

In line with the present study, it has been reported that eight weeks, five sessions per week running on treadmill significantly decreased TNF-α, CRP and IL-6 as well as increased VEGF and HIF-1α in cardiac muscle tissue of type 2 diabetic rats (19);

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However, there is still limited information on the effect of running on different slopes on inflammatory factors. For example da Rocha AL *et al.* compared the effect of running on positive and negative slopes on skeletal muscle of rat and reported that exercise in the negative slope increased cytokine levels and also running on the positive and negative slope increased IL-1β, IL-6 and IL-15 in muscle tissue (20). It seems that the reason for the differences in the results of this study with the present study is at the baseline levels and the method of measuring inflammatory factors, so that the rats in the present study had high levels of inflammatory factors in muscle tissue after AD induction. In a study eccentric contractions had more effects on increase of IL-6 levels and muscle injury than concentric contractions. However, concentric contraction also increased serum IL-6 levels (21). But the differences in these two studies were different in the statistical sample as well as the intensity and type of exercises. Cytokines appear to have different functions in muscle tissue that can even induce hypertrophy with adaptation to exercise (21). In a study, researchers also suggested that although eccentric exercises may induce muscle damage, nevertheless such trainings are now more widely used in rehabilitation because they can improve muscle strength and hypertrophy, by long- term adaptations. These researchers also reported that eccentric contractions with low recovery times caused excessive calcium release from the sarcoplasmic reticulum and increased reactive oxygen species as well as increased inflammatory receptor levels in muscle cells. But adapting to these exercises resulted in increasing the phagocytosis system and releasing proteolytic enzymes (such as elastase and myeloperoxidase), which results in the neutralizing and depleting phases of oxygenation, thereafter after cell clearance from reactive oxygen species (ROS) and inflammatory factors, the anti-inflammatory proteins accumulated in muscular cell and suppress the secretion of IL-6 and IL-1β (22). Therefore, exercise on negative slope in the present study appear to have more favorable effects on anti-inflammatory factors such as INF- γ in muscle tissue of rats with AD. However, considering the effect of training intervals, it seems that lack of evaluation of inflammatory cytokines at different times after exercise can be the limitation of the present study and it is suggested that this limitation should be considered in future studies. Also, considering the effect of the type of contraction on ROS, it seems that another limitations of the present study can be the lack of measurement of free radicals in the present study, therefore, it is suggested in future studies to measure oxidative stress levels in muscle tissue. Also as measure the behavioral memory test in the AD induced model is important, it is suggested that in future studies the researchers measure the behavioral memory test along with anti-inflammatory (IL-10 and TGF-β) genes in AD induced models.

Conclusion

Regarding to results of present study it can be concluded that AD induction can significantly increase the gene expression levels of TNF-α and INF- γ in the muscle tissue of rats nevertheless running on a positive and negative slope seems to improve TNF-α gene expression as well as running on positive slope can improve INF- γ gene expression in rats with AD.

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Conflict of interests

The authors declare that they have no conflict of interest.

Ethical approval: The study was approved by the Ethical Committee of Marvdasht Branch of Islamic Azad University.

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