Original Article

Changes in Aβ42, Neprilysin, and γ-Secretase in the Hippocampus of Male Rats Alzheimer's model: The Effects of Aerobic Training and Omega-3 Intake

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Abstract

Background: Alzheimer›s disease (AD) is characterized by excessive deposition of the amyloid-β peptide (Aβ) in the central nervous system and reducing its level is the goal of many medications. This study aimed to investigate the effect of aerobic training and omega-3 intake on Aβ42, neprilysin, and γ-secretase levels in the hippocampus of male rats Alzheimer›s model.

Methods: Fifty male Wistar rats (age: 12 weeks-old and weight: 222.31±11.91 g), were divided into the five groups including control Alzheimer's (AC), Alzheimer's with omega-3 intake (AO), Alzheimer's training (AT), Alzheimer's with omega-3 intake and training (AOT) and Healthy Control (HC). AD was induced by the injection of homocysteine (60mM) into the rat brain ventricle. Training on the treadmill with a speed of 20 m/min (60 minutes and 5 days/week) was applied. The supplement group received omega-3 supplement 800 mg/kg of body weight, daily for eight weeks. Levels of Aβ42, γ-secretase, and neprilysin protein were measured using ELISA method. In data analysis, one-way ANOVA and Tukey test as post hoc were used ($P<0.05$).

Results: The obtained results showed that the level of Aβ42 in the hippocampus of AC group was significantly higher than that of the HC group ($P=0.001$). Also, the level of Aβ42 in the hippocampus of AC group was significantly higher as compared to AO, AT, and AOT groups (P values 0.001, 0.007, and 0.003 respectively). The γ-Secretase level in the hippocampus of AC group was significantly higher than that in the HC group ($P=0.001$). Moreover, the γ-secretase levels in the hippocampus of the AC group were significantly higher compared to AO, AT, and AOT groups (P values: 0.002, 0.001, and 0.001 respectively). There was no significant difference in neprilysin levels of the hippocampus among the research groups ($P=0.534$).

Conclusion: It appears that exercise training and omega-3 consumption, can affect amyloidogenic pathways through reducing the level of γ-secretase, and lead to reduced level of hippocampus Aβ in AD subjects. Therefore, aerobic exercise training and omega-3 intake can be studied as a complementary therapy in Alzheimer's patients.

Keywords: Aerobic training, Omega-3 intake, Amyloid-β42, Neprilysin, γ-Secretase, Alzheimer's disease

Introduction

Alzheimer's disease (AD) is the most common form of dementia currently affecting over 50 million people worldwide, and over 5 million Americans (Alzheimer's Association). The prevalence of AD has increased greatly, particularly in countries with an increase in life expectancy. Prevalence is below 1% in the population under 60 years of age, increasing to 40% among those older than 85 (1).

An abnormal elevation of homocysteine (Hcy) level has been implicated as a marker for AD. Hyperhomocysteinemia is associated with increased cognitive decline in healthy older adults with a higher risk of cognitive impairment (2,3). In Farina et al study, cognitive status significantly declined over the follow-up period of the research (13 months) and that was paralleled by a significant increase in homocysteine

concentration (4). Mechanisms of increased levels of Hcy and its nervous toxicity effects are not fully understood, but a possible increase in Amyloid-β (Aβ) as the effect of increasing Hcy has been suggested (5,6).

The complex pathology of the disease is characterized by several hallmarks, such as prominent extracellular amyloid plaques (7). According to the amyloid cascade hypothesis, an alteration of Aβ metabolism is the central pillar of AD pathology and crucially influences and initiates other hallmarks (8). In AD, initial pathologic processes progress decades before the first cognitive symptoms appear in patients, a stage entitled preclinical Alzheimer's (9).

Aβ as a monomer is a very hydrophobic peptide that is found naturally in small amounts in the brain and has 37-49 amino acids which are created by Aβ precursor protein (AβPP) proteolysis (8,10,11). This peptide has no

fixed physiological function and as a result of a metabolic process, can produce both amyloidogenic and non-amyloidogenic products (12). If the AβPP, is cleaved by the enzyme α-secretase, Neuroprotective piece sAβPPα (a piece of APP by α-secretase isolated) is produced, which prevent the formation of Aβ plaques (13). However, if AβPP is cleaved by β- and then by γ-secretase, it increases the level of Aβ42 in CNS (14,15). Isolated parts in the β-secretase activity of AβPP, cause the production of sAβPPβ and finally γ-secretase activity on APP, followed by the production of Aβ. Aβ production in γ-secretase activity, is very susceptible to oligomerize and has extremely high toxicity (16), and the deposition of protein plaques in the brain is known as one of the main causes of early and important events in the pathogenesis of AD. Aβ is first formed in the hippocampus and is involved in the analysis of neurons in AD (10). A study on the transgenic rat (Tg) Alzheimer's, has strengthened this hypothesis that memory loss is associated with the level of Aβ (10,17). Researchers have shown that the injection of Aβ into the hippocampus of the brain, causes impaired learning and memory in rats, as well as neurodegeneration and neuronal dysfunction (18) and cleaning different areas of the brain from the presence of Aβ, can play an important role in improving the symptoms of AD (19).

According to the studies, some of the Aβ-degrading proteases help to adjust its level in the brain. These enzymes are mainly serine or metalloproteinase which include an insulin-degrading enzyme, neprilysin, endothelin converting enzyme, angiotensin-converting enzyme, and matrix metalloproteinase-9. Among these factors, neprilysin is the main degrading enzyme of Aβ (20).

The main modifiable risk and protective factors for AD are socioeconomic factors such as level of education, lifestyle factors such as alcohol and tobacco consumption and physical activity, as well as dietary factors such as the consumption of caffeine, antioxidants, and fatty acids (1,21).

Omega-3 fatty acids are essential fatty acids that are the components of neuronal cell membranes in the brain (22-24). Several studies have highlighted the neuroprotective roles of Omega-3 in neurodegenerative disorders such as Huntington's disease (25) and Parkinson's disease (21), as well as AD and mild cognitive impairment (26,27). Also, it is asserted that physical activity plays a pivotal role in the prevention of neurodegenerative disorders. Although age is a dominant risk factor for AD, epidemiological studies have shown that exercise may significantly decrease age-related risks for AD (28-31).

Because of the shared neurobiological and physiological effects of physical activity (PA) and omega-3 intake, several human and animal studies have speculated about the additive or multiplicative benefits that might arise from combining omega-3 intake with PA (32,33).

For example, PA may provide an avenue by which the effects of docosahexaenoic acid (DHA) on cellular integrity and cognitive function are enhanced (33,34). The combination of PA and DHA intake have additive effects on synaptic plasticity and membrane structure biomarkers in the dentate gyrus of the hippocampus, such that mice receiving both DHA and PA had greater levels of synaptic proteins than their counterparts not receiving PA (35). However, these effects were not mirrored behaviorally. Instead, physical inactivity without DHA supplementation resulted in impaired learning compared to DHA intake, PA, or both (35). Studies in humans have not yet examined whether DHA levels moderate the effect of PA on cognitive performance in a similar way to that demonstrated in rodents.

Overall, because the effect of omega-3 intake along with aerobic training on Aβ metabolism has not been well studied, this study aimed to investigate the effect of aerobic training and omega-3 intake on the hippocampus levels of Aβ42, neprilysin, and γ-secretase of male rats Alzheimer's model.

Material and Methods

In this experimental study conducted in the laboratory method, 50 head of adult male Wistar rats with a weight range of 100 to 150 g and the age of 8 weeks prepared from Pasteur Institute in northern Iran were used. The rats were kept in an environment with a temperature of $22 \pm 2°C$, humidity of $50 \pm 5\%$, and the light-dark cycle of 12:12 hours in polycarbonate cages (5 rats per cage). After 4 weeks, 40 rats were selected for intracerebroventricular (ICV) Injection of Hcy and 10 rats were selected as the healthy group.

Intracerebroventricular injection of Hcy

When the rat became 200 to 250g in weight, it was anesthetized by intraperitoneal injection of ketamine and xylazine (with doses of 50 and 4 mg/kg). The head of rats was placed into stereotaxic surgery and according to Paxinos and Watson atlas, within the context of the brain, the cannula was inserted and connected to the skull with dental cement. A week later connecting a cannula, Hcy solution (1 μL) was injected into the brain ventricles by Hamilton syringe. An effective amount of Hcy for neural degeneration and Alzheimer's 0.6 M (0.86 μg per mouse) was designated (36).

The shuttle box was used to assess behavioral change and ensure the induction of AD. Rats were randomly divided into the five groups including control Alzheimer's (AC), Alzheimer's with omega-3 intake (AO), Alzheimer's with training (AT), and Alzheimer's with omega-3 intake and Alzheimer training (AOT), and also a group as Healthy Control (HC). Because 6 head of rats died after Cannula or during the study period, the number of samples at the end of the study was 44 head.

Aerobic training protocol

Aerobic training included 5 days a week for an entire period of 8 weeks and was conducted in 3 stages. In the familiarization phase (first week) the rats walked every day for 10-15 minutes on a treadmill at a speed of 10 m/min. At the overload (the second and third weeks), gradually during the second week, the intensity and duration of the training were increased to the final stage of 60 minutes with a speed of 20 m/min. In the process of preservation or stabilization (fourth to the eighth week), training continued with the same intensity until 8 weeks ended. This is equivalent in intensity to 50% to 55% of maximum oxygen consumption in a rat (37,38). This training protocol has already been used in a similar work by the researcher.

Omega-3 intake

Omega-3 receiving groups received 800 mg/kg omega-3 supplement daily by gavage for eight weeks (39). The omega-3 supplement used included 136 mg/ml DHA and 139 mg/mL EPA (40).

All subjects, 72 hours after the last training session, were anesthetized with a combination of intraperitoneal injection of ketamine (50 mg/kg) and Xylazine (4 mg/kg) (36). To collect samples of the hippocampus, the subjects were isolated in the neck by cutting pliers, using the knife, the skull was split and the brain was removed with caution. Healthy brain by the surgery was split exactly in half and given the coordinates of the hippocampus using Paxinos atlas, the hippocampus was removed from the limbic system. Hippocampus samples collected for subsequent measurements were stored at -80°C. It should be noted, all procedures including insertion of the cannula, AD inducing, the training protocol and killing and biopsy procedures were done according to the regulations of the biological research ethics committee of Mazandaran University.

To measure hippocampal levels of Aβ42, γ-secretase, and neprilysin, initially 50 mg hippocampus tissue was placed in cold saline citrate buffer solution. Then, the tissue was homogenized by micro-homogenizer for 10 minutes. The homogeneous tissue was centrifuged and the supernatant was transferred into Eppendorf. This product was used to measure the level of Aβ42, γ-secretase, and neprilysin in hippocampal tissue. Hippocampus Aβ42 levels were measured by ELISA using research kits for rats (manufactured by Cusabio Biotech Wuhan, China) and following the manufacturer's instructions. The sensitivity of the measuring kit was 0.225 pg/mL and the coefficient of variation was 8.20%. neprilysin hippocampus level was measured by ELISA using research kits for rats (manufactured by Wuhan Cusabio Biotech) according to the manufacturer's instructions. The sensitivity of the measuring kit was 11.75 pg/mL and the intra-coefficient was 8.70%. The level of hippocampus γ-Secretase was measured by ELISA using research kits for rats (manufactured by Sunlong, China) and following the manufacturer's instructions. The sensitivity of the measuring kit was 0.60 pg/mL and the coefficient of variation within the test was 7.40%.

Shapiro-Wilk test was used to assess the normality of the data and the Levene's test was used to check equal variances. After the assumption of the normal distribution and equality of variances, ANOVA and Tukey test were used for statistical analysis and data comparison between groups, and the Pearson correlation coefficient was used to examine relationships between variables. All statistical calculations were performed using SPSS 23 statistical software at a significant level of $P < 0.05$.

Results

Table 1 shows the mean and standard deviation of rats' weight before and after 8 weeks of aerobic training in the research groups.

One-way analysis of variance showed that there was no significant difference between the weight of the rats before ($P = 0.456$, F = 0.968) and after ($P = 0.446$, F = 0.983) training courses in all groups.

After verification of normality and using one-way analysis of variance, a significant difference in the latency to enter the dark area was observed among groups ($P = 0.001$, F = 19.21). The findings from the post hoc test comparing pair's latency to enter the dark compartment in different groups showed that the index at baseline in the group of healthy control was significantly higher than that in the Alzheimer's groups ($P = 0.001$). There was no significant difference in the latency to enter the dark area among Alzheimer's groups ($P > 0.05$).

Table 2 shows the comparison of the effects of aerobic training and omega-3 intake on the levels of Aβ42, γ-secretase and neprilysin in the research groups. As it is seen, there is a significant difference in the mean levels of Aβ42 and γ-secretase in the study groups (P values

Table 1. Mean and standard deviation of rat's weight before and after 8 weeks of aerobic training in the research groups *

Research groups	Initial weight (g)	Final weight (g)	Arrival to dark areas (S)
HC	217.13 ± 22.90	325.38 ± 11.90	183.82 ± 78.29
AC	220.9 ± 63.90	301.24 ± 25.90	25.21 ± 50.78
AO	227.11 ± 25.97	303.29 ± 13.51	23.18 ± 75.20
AT	221.11 ± 33.70	327.44 ± 33.90	23.15 ± 44.96
AOT	227.11 ± 63.90	307.31 ± 38.70	25.25 ± 0.52

* Numbers are expressed as the mean ± standard deviation.

Table 2. Comparison of Aβ42, γ-secretase and Neprilysin levels (mean ± SD) in the hippocampus of different groups and the results of the analysis of variance *

Group	Aβ$_{42}$ (pg/mg tissue)	γ-Secretase (pg/mg tissue)	Neprilysin (ng/mg tissue)
HC	48.33 ± 16.98	46.50 ± 8.15	7.19 ± 1.72
AC	102.01 ± 20.73	87.03 ± 18.62	7.48 ± 1.31
AO	59.87 ± 18.91	58.97 ± 13.35	9.45 ± 3.07
AT	70.22 ± 24.50	37.02 ± 8.29	8.72 ± 2.44
AOT	66.25 ± 15.13	42.96 ± 15.47	9.65 ± 3.66
F	8.466	21.829	0.744
P	0.001[a]	0.001[a]	0.534

* Numbers are expressed as the mean ± standard deviation.
[a] Significant difference between groups ($P < 0.05$).

0.001 and 0.001 respectively), but there was no significant difference in the mean of neprilysin among the research groups ($P = 0.534$).

Aβ42 level of the hippocampus
As it is seen in Figure 1, the level of Aβ42 in the hippocampus of AC was significantly higher compared to the HC group ($P = 0.001$). Also, the level of the Aβ42 in the hippocampus of AC group was significantly higher than the same values in the AO, AT, and AOT groups (P values 0.001, 0.007, and 0.003 respectively). On the other hand, there was no significant difference in this item among AO, AT, and AOT groups ($P > 0.05$). Figure 1 shows the results of comparing the levels of hippocampus Aβ42 in the studied groups after 8 weeks of Aerobic training and omega-3 intake.

γ-Secretase level of the hippocampus
The results showed that the γ-secretase level in the hippocampus of the AC group was significantly higher than that in the HC group ($P = 0.001$). Also, the γ-secretase level in the hippocampus of the AC group was significantly higher compared to the AO, AT, and AOT groups (P values, 0.002, 0.001, and 0.001 respectively). γ-Secretase level in the hippocampus of the AO group was significantly higher than that in the AT group ($P = 0.019$). But, there was no significant difference in hippocampus γ-Secretase levels between AT and AOT groups ($P = 0.848$). Figure 2 shows the results of comparing the levels of hippocampus γ-secretase of research groups after 8 weeks of aerobic training and omega-3 intake.

Neprilysin level of the hippocampus
Based on the data presented in Table 2, there was no significant difference in the neprilysin level of the hippocampus among research groups ($P = 0.534$). Figure 3 shows the results of comparing the levels of hippocampus neprilysin of research groups after 8 weeks of aerobic training and omega-3 intake.

Discussion
We found that ICV Injection of Hcy is associated with increased levels of Aβ42 and γ-secretase in the hippocampus. Also, aerobic training and omega-3 intake lowered Aβ42 and γ-secretase levels of the hippocampus in AD subjects.

It has been well established that elevated plasma homocysteine and disturbed homocysteine metabolism are risk factors for AD (1). However, the exact pathophysiological mechanisms linking high homocysteine levels with AD have not been cleared yet. Several potential mechanisms resulting in harmful effects of this amino acid in the brain have been proposed, including oxidative stress (41), cerebrovascular damage (42), DNA damage (43), and activation of N-methyl-D-aspartate receptors (44). Several studies showed that disturbed homocysteine metabolism is related to increased CSF levels of sAPP forms and Aβ42, and may contribute to the accumulation of amyloid pathology in the brain through increasing γ-secretase pathway (43,45). For example, Lin et al showed that Hcy increases the production of Aβ possibly by increased expression of APP, as well as induction of hypomethylation of APP and PS1 gene promoters (46); whereas in rats, hyperhomocysteinemia increases cerebral Aβ production by phosphorylation of amyloid precursor protein and enhancing expression of γ-secretase (47). More recently, mice with diet-induced hyperhomocysteinemia were shown to have elevated brain Aβ levels and amyloid deposition and it was suggested that this association is mediated by the activation of the γ-secretase pathway (48). These previous reports provide a possible explanation of the biochemical process, connecting disturbed homocysteine metabolism and increased CSF levels of sAPP forms and Aβ42.

Based on the evidence, both microglia and astrocytes secret Aβ protein (49). Senile plaques, mainly composed of peptides Aβ, have been widely proven in the pathogenesis of AD (49,50). In particular, increased level of Aβ deposition in plaques outside the cell causes synaptic dysfunction, neuronal network dysfunction, mitochondrial dysfunction, neuronal cell death, and memory loss (49,51,52). Although the mechanism of neurotoxicity caused by Aβ is not yet clear, but it has
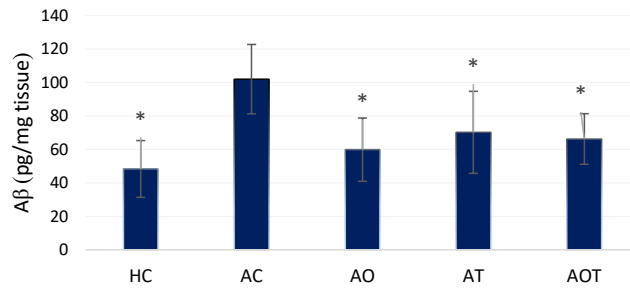
Figure 1. Aβ42 levels in the hippocampus of the studied groups. * Significant difference compared to the AC group ($P<0.05$)
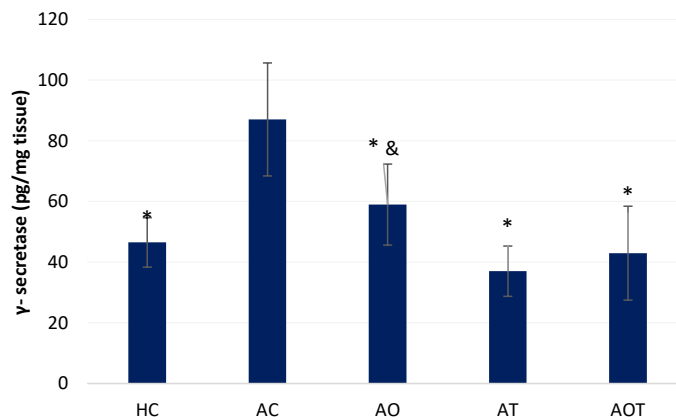

Figure 2. γ-Secretase levels in the hippocampus of the studied groups. *Significant difference compared to the AC group. & Significant difference compared to the AT group ($P<0.05$)
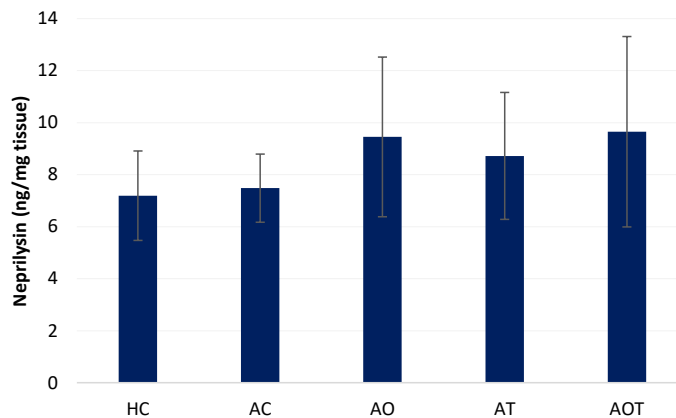

Figure 3. Neprilysin levels in the hippocampus of the studied groups

been widely proven that the accumulation of Aβ peptide in the brain causes induction of oxidative stress and neuroinflammation (49,50). In vitro studies have shown that the injection of Aβ42 in primary hippocampal neurons leads to increased planting in the indices of oxidative stress and neurotoxicity (53,54). This peptide associated with oxidative stress, in this regard adding vitamin E as an antioxidant, significantly dampens the effects of oxidative stress and neurotoxicity induced by Aβ42 (53). It has been indicated that Aβ40 injection into

the brain of rats is associated with the induction of free radical damage and changes in antioxidant defense such as glutathione depletion in the prefrontal cortex and hippocampus of rats (55).

It has been shown that Aβ acts as an inflammatory agent and causes inflammatory mediators, such as cytokines, to be activated in brain and, as a result, increases the risk of Alzheimer's. Besides, the presence of activated microglia and astroglia around senile plaques supports the role of Aβ in inflammation. Microglia and astrocytes may have

a neuroprotective role by swallowing and clearing Aβ aggregates in the brain (56,57). Although these aggregates can mediate neurotoxicity effects through the release of pro-inflammatory cytokines, chemokines, ROS, and protein supplements (56). Besides, APP expression by IL-1 (as an inflammatory cytokine) increases and thus enhances amyloidosis and leads to a vicious cycle (58). Now, there is a growing evidence that low to moderate-intensity training is an important factor in neural degenerative diseases (59).

In the present study, Aβ42 level in the hippocampus of AO, AT, and AOT groups was significantly lower than AC group, without any significant differences between AT, AO, AOT, and HC groups.

According to the amyloid cascade hypothesis and the law of mass action, synthesis and degradation regulate the level of proteins, including Aβ42 (16).

In the present study, to investigate changes in Aβ production in the training groups, the γ-secretase was evaluated and it was found that its level in the AC group was significantly higher than that in the HC group. Thus increasing Aβ42 levels in the hippocampus of Alzheimer's rat compared to a healthy rat can be caused by increased levels of γ-secretase in hippocampus.

The results of this study showed that the levels of γ-secretase in the hippocampus of AT and AOT groups were significantly lower than that in the AC group. Also, there was no significant difference in hippocampus neprilysin levels of the studied groups. As mentioned before, neprilysin is considered as the main Aβ degrading enzyme and as a regulator has raised concentrations of Aβ in the brain functional surfaces (60,61). Failure to raise the level of neprilysin means no change in the demolition/clean Aβ42 from the hippocampus of Alzheimer's subjects which seeks to increase the level of the index and can lead to the development of AD risk. Kang and Cho examined the effect of 6 weeks of treadmill training on insulin signaling and brain Aβ levels in the streptozotocin-induced Alzheimer in rats. Their results showed a significant decrease in Aβ42 levels and increase of insulin signaling in the brains of Alzheimer training group compared to Alzheimer control group. They suggested that reducing insulin signaling is associated with elevated levels of γ-secretase, which leads to an increase of Aβ and improves insulin signaling caused by six weeks of training on a treadmill, might be a moderation of γ-secretase to reduce Aβ (62). Also, Liu et al showed that the number and size of Aβ plaques in the hippocampus of a rat with AD, five months after the treadmill training, was significantly reduced. The levels of Aβ42, tau protein, and PS1 expression decreased significantly as a result of training on the treadmill. Besides, reductions in the levels of CTFs and sAβPPβ in training transgenic rats were observed. The researchers concluded that perhaps treadmill workouts prevent the amyloidogenic pathway

and increases the likelihood of APP degradation through non-amyloidogenic pathway (63). Also, Kang et al stated that 12 weeks of treadmill training prevented the disorder gene mutation PS2 and reduced the accumulation of Aβ by inhibiting the activity of β- secretase and its products (64). Besides, Um et al showed that 16 weeks of treadmill training causes a significant decrease in Aβ42 in the brain of rats with Alzheimer's (65). They also showed that training with two different intensities, through reducing the γ-secretase as an amyloidogenic pathway, causes a decrease in Aβ, which is consistent with the results obtained in the present research.

There are several theories about changes in Aβ levels and metabolism as the result of training. The physical activity regulates protein level both at the mRNA and/or protein stage, which induce anatomical, chemical and electrophysiological change in nerves, and enhance the plasticity of neurons (66). For example Adlard et al stated that exercise training can probably mediate the metabolism of APP and Aβ cascade in the brain to reduce the production of Aβ (decreasing amyloidogenic activity) which is independent of the neprilysin and insulin-degrading enzyme (67). The second possibility is that exercise directly modulates the APP metabolism by increasing the activity of neurons. For example, processing of APP can be completed by mitogen-activated protein kinase (MAPK) and phospholipase C and it has been proved that these pathways are activated through exercise (68). On the other hand, physical activity increases cholinergic activity which is involved in neuronal plasticity (66). It has been mentioned that exercise can probably improve behavioral disorders by reducing the number of peptide Aβ42 through increasing the production of neurotrophic factors (NGF, BDNF, and IGF-1) which are important for neuronal survival and proliferation of neuronal and synaptic plasticity (69). Also, physical activity reduces Aβ plaque and improves spatial learning (three-dimensional), memory, synaptic plasticity, and nerve tissue of AD in rat (70). Several studies have reported significant decrease of the cytoplasmic surface of apoptotic markers such as cytochrome C, caspases-9, caspases-3, and Bax protein in the brains of active Alzheimer's rat model compared to the inactive Alzheimer's rat model. In Alzheimer's rat model, decreased pro-apoptotic proteins, including cytochrome C and boxes with physical activity, possibly by preventing apoptotic pathways related to caspases, are systematically associated with lower levels of protein Aβ (65,71). However, Park et al postulated a cyclic process that stimulates Aβ in inflammation and suggested that signaling TNF-α, ultimately leads to the production of Aβ peptides causing production of new pathogenic Aβ peptides that increase its production and thus leads to AD that can create a stronger cycle (72). In this regard, Nichol et al demonstrated that inflammatory markers (IL-1β and

TNF-α) in the hippocampus of Alzheimer's transgenic rat was higher compared to the healthy rat and the levels of anti-inflammatory agents (IFN-γ and MIP-1α) were lower in Alzheimer's transgenic rat than those in the healthy rat. After 3 weeks of training, the levels of IL-1β and TNF-α decreased and were close to the normal group that this reduction was associated with an increase in IFN-γ and MIP-1α. Also a significant decrease in the levels of Aβ40 solution and fibrillar Aβ solution in the cortex of Alzheimer's transgenic rat after 3 weeks optional practice was observed (73). Also, in Um et al study, the SOD-1 protein and catalase in the brains of active Alzheimer's rat models showed a significant increase compared to the inactive ones. Exercise causes increased levels of these defense indexes that these changes are associated with reduced apoptotic protein (cytochrome C, caspases-9, caspases-3, and bax) and an increase in Hsp70 and BDNF which is induced by regular physical activity in the brain and then was mediated by peptides Aβ42 clinically reduced in rat with AD (65). It has been shown that 16 weeks of treadmill exercise combined with α-lipoic acid, decreased levels of brain Aβ42 in transgenic Alzheimer's rat model. The researchers reported that increased oxidative stress, is one of the main factors involved in Alzheimer's which leads to increased production of ROS and causes the destruction of cellular structures, and ultimately apoptosis increases the production of Aβ. Exercise alone and in combination with α-lipoic acid supplementation results in increased levels of oxidative stress and antioxidants as immunosuppressive agents and finally leads to reduced apoptotic index and Aβ (71). Possible mechanisms in this regard include the reduction of oxidative stress and increase of antioxidant defense enzyme activity, which enhance the α-secretase and inhibit β- and γ-secretase. That is, the processing of APP is conducted to non-amyloidogenic pathway (74). The improvement in Aβ and γ-secretase levels in the present study may also be due to the reduction of oxidative stress and the improvement of antioxidant defense through omega-3 supplementation and aerobic exercise.

Conclusion

However, many researchers have proposed using different drugs for the treatment of AD, each has serious side effects. Thus, lifestyle changes such as exercise and nutrition can be used as a complementary method to the medical treatment and also lead to a reduction in the side effects of high doses of medication. Since many drug treatment efforts for Alzheimer focus on inhibiting γ-Secretase and reduction of Aβ and according to the survey results, it appears that exercise training and omega-3 consumption, can prevent amyloidogenic pathways by reducing the level of γ-Secretase , and lead to reducing the level of hippocampus Aβ of AD subjects. In total, aerobic exercise training and omega-3 intake can be studied as complementary therapy in Alzheimer's patients.

One of the limitations of this study was the absence of the shuttle box test in the post-test. Therefore, it is suggested that this functional test be performed in future researches.

It is suggested that the effect of omega-3 supplementation and exercise training on Aβ transmitting factors, from blood brain barrier, be investigated.

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