The Effects of Aerobic Training and Omega-3 Intake on A β 42, Neprilysin, and γ -Secretase in the Hippocampus of Male Rats Alzheimer's model

Ali Yaghoubi (✉ yaghoubiali65@gmail.com)

Islamic Azad University

Research

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Abstract

Alzheimer's disease (AD) is characterized by excessive deposition of the amyloid- β peptide ($A\beta$) in the central nervous system and reducing its level is the goal of many medications. Thus this study aimed to investigate the effect of aerobic training and omega-3 intake on $A\beta_{42}$, Neprilysin, and γ -secretase in the hippocampus of male rats Alzheimer's model. 50 male wistar rats (12weeks-old and weight 222.31 ± 11.91 g), divided into 5 groups including, control Alzheimer's (AC), Alzheimer's with the omega-3 intake (AO), Alzheimer training (AT), Alzheimer's with omega-3 intake, and Alzheimer training (AOT) and Healthy Control (HC). Alzheimer's disease induced by injection of homocysteine (60mM) into the rat brain ventricle. Training with a speed of 20 m/min (60 minutes and 5 days/week on the treadmill) was applied. The supplement group was received an Omega-3 supplement 800mg/kg of body weight, daily for eight weeks. Level of $A\beta_{42}$, γ -secretase, and Neprilysin protein measured using ELISA method. In data analysis, a one-way ANOVA and Tukey test as a post hoc were used ($P < 0.05$). The obtained results showed that the level of $A\beta_{42}$ in the hippocampus of AC was significantly higher than the HC group ($P = 0.001$). Also, the level of the $A\beta_{42}$ in the hippocampus of AC was significantly higher than AO, AT, and AOT groups (P values 0.001, 0.007, and 0.003 respectively). Also the γ -Secretase level in the hippocampus of AC was significantly higher than the HC group ($P = 0.001$). Also, the γ -secretase levels in the hippocampus of the AC group were significantly higher than AO, AT, and AOT groups (P values, 0.002, 0.001, and 0.001 respectively). There was no significant difference between Neprilysin levels of the hippocampus of research groups ($P = 0.534$). 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 $A\beta$ hippocampus of Alzheimer's disease subjects. In total, aerobic exercise training and omega-3 intake can be studied as complementary therapy in Alzheimer's patients.

1. 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 that have seen 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) levels 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 this regard, Farina et al. (2017) showed that cognitive status significantly declined over the follow-up period of research (13 months) and that was paralleled by a significant increase in homocysteine concentrations [4]. Mechanisms of increased levels of Hcy and creation of its nervous toxicity effects are not fully understood, but a possible increase in Amyloid- β ($A\beta$) in the effect of increasing Hcy 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 $A\beta$

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].

A β 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 A β precursor protein (A β PP) 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 A β PP is cleaved by the enzyme α -Secretase, Neuroprotective piece sA β PP α (a piece of APP by α -secretase isolated) produced and prevent the formation of A β plaques [13]. Conversely, if fragmentation is needed for successive APP, first by β - and then by γ -secretase, followed by the formation of A β 42 and causes an increase in the level of A β 42 in the nervous system [14, 15]. Isolated parts in the β -secretase activity of A β PP, causes the production of sA β PP β and finally γ -secretase activity on APP, followed by the production of A β . A β 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 that the first to be 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 A β [10, 17]. Researchers have shown that A β injected into the hippocampus of the brain, causing impaired learning and memory in rats and causes neurodegeneration and neuronal dysfunction [18] and cleaning different areas of the brain from the presence of A β , can play an important role in improving the symptoms of AD [19].

According to the studies, some of the A β -degrading proteases help to adjust its level in the brain. These enzymes are mainly serine or metalloproteinase which include an Insulin-Degrading Enzyme (IDE), Neprilysin (NEP), Endothelin Converting Enzyme (ECE), Angiotensin-Converting Enzyme (ACE), and Matrix Metalloproteinase-9 (MMP-9). Among these factors, Neprilysin is the main degrading enzymes of A β [20].

The main modifiable risk and protective factors for AD are socioeconomic factors such as level of schooling; lifestyle factors such as alcohol and tobacco consumption and level of physical activity; and 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 (HD) [25] and Parkinson's disease (PD) [21] as well as AD and Mild Cognitive Impairment (MCI) [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 [32, 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 have 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 mice with 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 A β metabolism has not been well studied. Thus, this study aimed to investigate the effect of aerobic training and omega-3 intake on the level of A β 42, Neprilysin, and γ -secretase Alzheimer's hippocampus of male rats Alzheimer's model.

2. Material And Methods

The current study is experimental which was conducted in the laboratory method. In this study, 50 head of adult male Wistar rats with a weight range of 100 to 150 g and the age of 8 weeks were used which was prepared by the Pasteur Institute in northern Iran. The rats were kept in an environment with a temperature of $22 \pm 2^\circ\text{C}$, humidity $50 \pm 5\%$, 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 for the healthy group.

Intracerebroventricular (ICV) Injection of Hcy

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

The shuttle box was used to assess behavioral change and ensure the induction of Alzheimer's disease. Alzheimer's rat was divided randomly into 5 groups including control Alzheimer's (AC), Alzheimer's with the omega-3 intake (AO), Alzheimer training (AT), and Alzheimer's with omega-3 intake and Alzheimer training (AOT), there was also a group called 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 the entire period of 8 weeks and 5 days a week and was conducted in 3 stages. In the trading phase (first week) the rats walk 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, added to the

intensity and duration of the training, has reached the final 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 to 8 weeks ended. This is equivalent intensity by 50 to 55 percent of maximum oxygen consumption in a rat [37, 38].

Omega-3 intake

Omega-3 receiving groups, intake 800 mg/kg of the 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, with a combination of intraperitoneal injection of Ketamine (50 mg per kg) and Xylazine (4 mg per kg) were anesthetized [36]. To collect samples of the hippocampus, the subjects were isolated in the neck by cutting pliers, using the knife, split the skull and brain were 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 consisted of a cannula, inducing AD inducing, the training protocol and killing and biopsy were done according to the regulations of the biological research ethics committee of Mazandaran University.

To measure hippocampal levels of A β 42, γ -secretase, and Neprilysin, initially 50 mg hippocampus tissues were placed in cold saline citrate buffer solution. Then the tissue was homogeneous by micro-homogenizer for 10 minutes. By homogeneous tissue was centrifuged and the supernatant was transferred into eppendorf. This product was used to measure the level of A β 42, γ -secretase, and Neprilysin in hippocampal tissue. Hippocampus A β 42 levels were measured by ELISA using research kits for rats (manufactured by Cusabio Biotech Wuhan, China) and following the manufacturer's instructions. The sensitivity measurement kit was 0.225 pg/mL and the coefficient of variation was 8.20 percent. Neprilysin hippocampus levels were measured by ELISA using research kits for rats (manufactured by Wuhan Cusabio Biotech) according to the manufacturer's instructions. The sensitivity measurement kit was 11.75 pg/mL and the intra-coefficient was 8.70 percent. The level of γ -secretase hippocampus were measured by ELISA using research kits for rats (manufactured by Sunlong, China) and following the manufacturer's instructions. The sensitivity measurement kit was 0.60 pg/mL and the coefficient of variation within the test was 7.40 percent.

Shapiro-Wilk test was used to assess the normality of the data and the Leven 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 SPSS23 statistical software at a significant level of $P \leq 0.05$.

3. Results

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

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.			

After verification of normality and using one-way analysis of variance showed that there was no significant difference between the weight of the rats in the first ($P = 0.456$, $F = 0.968$) and end ($P = 0.446$, $F = 0.983$) training courses between groups.

After verification of normality and using one-way analysis of variance showed that there is a significant difference between the latency to enter the dark area between 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 upper than Alzheimer's group ($P = 0.001$). There was no significant difference between the latency to enter the dark area between groups between Alzheimer's group ($P > 0.05$).

Table 2 showed the test results to compare the effects of aerobic training and omega-3 intake on the levels of A β 42, γ -secretase, Neprilysin in the research groups. The results in Table 2, there was a significant difference between the average levels of A β 42 and γ -secretase in study groups (P values 0.001 and 0.001 respectively), but there was no significant difference between the mean of Neprilysin in the research groups ($P = 0.534$).

Table 2
Comparison of index levels of the study (mean $\pm$ SD) in the hippocampus of study groups and the results of the analysis of variance *

Group	A β ₄₂)pg/mg tissue(	γ - secretase)pg/mg tissue(	Neprilysin)ng/mg tissue(
HC	48.33 $\pm$ 16.98	46.50 $\pm$ 8.15	7.19 $\pm$ 1.72
AC	102.01 $\pm$ 20.73	87.03 $\pm$ 18.62	7.48 $\pm$ 1.31
AO	59.87 $\pm$ 18.91	58.97 $\pm$ 13.35	9.45 $\pm$ 3.07
AT	70.22 $\pm$ 24.50	37.02 $\pm$ 8.29	8.72 $\pm$ 2.44
AOT	66.25 $\pm$ 15.13	42.96 $\pm$ 15.47	9.65 $\pm$ 3.66
F	8.466	21.829	0.744
P	0.001&	0.001&	0.534
* Numbers are expressed as the mean $\pm$ standard deviation.			
& existing a significant difference (P $\leq$ 0.05) between the study groups.			

A β 42 level of the hippocampus

Information available in Fig. 1 shows the level of A β 42 in the hippocampus of AC was significantly higher than the HC group (P = 0.001). Also, the level of the A β 42 in the hippocampus of AC was significantly higher than AO, AT, and AOT groups (P values 0.001, 0.007, and 0.003 respectively). On the other hand, there was no significant difference between AO, AT, and AOT groups (P > 0.05). Figure 1 shows the results of research comparing the levels of A β 42 hippocampus 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 AC was significantly higher than the HC group (P = 0.001). Also, the γ - secretase levels in the hippocampus of the AC group were significantly higher than AO, AT, and AOT groups (P values, 0.002, 0.001, and 0.001 respectively). γ - secretase levels in the hippocampus of the AO group were significantly higher than AT group (P = 0.019). But there was no significant difference between hippocampus γ - secretase levels in AT and AOT group (P = 0.848). Figure 2 shows the results of research comparing the levels of γ - secretase hippocampus 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 between the Neprilysin level of the hippocampus of research groups (P = 0.534).

4. Discussion

We found ICV Injection of Hcy is associated with high A β 42 and γ -Secretase levels of the hippocampus. Also, aerobic training and Omega-3 intake lowered A β 42 and γ -Secretase levels of the hippocampus in AD subjects.

It is 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 are not yet clear. 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 showed that disturbed homocysteine metabolism is related to increased CSF levels of sAPP forms and A β 42, and may contribute to the accumulation of amyloid pathology in the brain through increasing γ -secretase pathway [43, 45]. For example, Lin et al (2009) show that Hcy increases the production of A β 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 A β 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 A β 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 A β 42.

Based on the evidence, both microglia and astrocytes secreted A β protein [49]. Senile plaques, mainly composed of peptides A β is that its main role has widely proven in the pathogenesis of Alzheimer's disease [49, 50]. In particular, increased levels of A β deposition in plaques outside the cell and cause synaptic dysfunction, neuronal network dysfunction, mitochondrial dysfunction, neuronal cell death, and memory loss [49, 51, 52]. Although the mechanism of neurotoxicity caused by A β is not yet clear, but widely proven that the accumulation of A β peptide in the brain causes induction of oxidative stress and neuroinflammation [49, 50]. In vitro studies have shown that injection of A β 42 in primary hippocampal neurons leads to increased planting in the indices of oxidative stress and neurotoxicity [53, 54]. This peptide associated with oxidative stress caused by A β , adding vitamin E as an antioxidant, significantly dampens the effects of oxidative stress and neurotoxicity induced A β 42 [53], which suggests that the ability of the peptide A β 42-induced neurotoxicity toxic oxidative stress-mediated. This regard indicated that A β 40 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 A β acts as an inflammatory agent and causes inflammatory mediators such as cytokines and complement components activated brain areas to increase the risk of Alzheimer's. Besides, the presence of activated microglia and astroglia around senile plaques supports the role of A β in inflammation. Microglia and astrocytes may have a neuroprotective role by swallowing and clearing A β

aggregates in the brain [56, 57]. Although it 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) is increased and thus enhancing amyloidosis and leads to a vicious cycle [58]. Now, there is growing evidence that low to moderate-intensity training is an important factor for neural degenerative diseases [59].

In the present study A β 42 levels in the hippocampus of AT group were significantly lower than the AC group and no difference was observed between the levels of the A β 42 hippocampus of AT, AO, AOT, and HC groups.

The amyloid cascade hypothesis and the law of mass action, each level balance between the synthesis and degradation of proteins in the body/cleans it and A β levels in the brain balance between the synthesis and degradation/clean it [16].

In the present study to study changes in A β production in the training groups, the γ -secretase was evaluated and it was found that the level of γ -secretase of AC group was significantly higher than HC group. Thus increasing A β 42 levels in the hippocampus of Alzheimer's rat compared to a healthy rat can be caused by increased levels of γ -secretase in hippocampal them.

The results of this study showed that the level of γ -secretase in the hippocampus of AT and AOT was significantly lower than the AC group. The results showed that there was no significant difference between Neprilysin hippocampus levels of research groups. As mentioned before, Neprilysin is considered as the main degrading enzyme A β and as a regulator has raised concentrations of A β in the brain functional surfaces [60, 61]. Failure to raise the level of Neprilysin means no change in the demolition/clean A β 42 from the hippocampus of Alzheimer's subjects which seeks to increase the level of the index and can lead to the development of Alzheimer's disease risk. Kang and Cho (2014) examined the effect of 6 weeks of treadmill training on insulin signaling and brain A β levels in the Alzheimer induce rat by streptozotocin. Their results showed a significant decrease in A β 42 levels and increase insulin signaling in the brains of rat of Alzheimer training compared to Alzheimer control groups. They suggested that reducing insulin signaling is associated with elevated levels of γ -secretase, which leads to an increase in A β and improves insulin signaling caused by six weeks of training on a treadmill, might be a moderation of γ -secretase to reduce A β [62]. Also, Liu et al (2013) showed that the number and size of A β plaques in the hippocampus of a rat with Alzheimer's disease five months after the treadmill training was significantly reduced. The levels of A β 42, tau protein, and PS1 expression decreased significantly as a result of training on the treadmill. Besides, a reduction in the levels of CTFs and sA β PP β in training transgenic rats was observed. The researchers concluded that perhaps treadmill workouts prevent the amyloidogenic pathway and the breakdown of APP to be adjusted to non-amyloidogenic[63]. Also, Kang et al (2013) stated that 12 weeks of treadmill training to prevent the disorder gene mutation PS2 and reduced the accumulation of A β by inhibiting the activity of β -secretase and its products [64]. Besides, Um et al (2008) also showed that 16 weeks of treadmill training causes a significant decrease in A β 42 in the brain of rats with Alzheimer's protein levels [65]. The results also showed that training with two

different intensities by reducing the γ -secretase that is amyloidogenic pathway causes a decrease in A β that is consistent with the results obtained from the research.

There are several theories about changes in A β levels and metabolism (synthesis and degradation) as a result of training. According to the physical activity mediates many gene products both at the mRNA and protein level, Inducer of anatomical changes, chemical and electrophysiological nerve, is enhancer the plasticity of neurons [66], multiple paths are likely enabled to adjust the level of A β directly or indirectly. Adlard et al (2005) stated that exercise training can probably mediate the metabolism of APP and A β cascade in the brain to reduce the production of A β (decreasing amyloidogenic activity) which is independent of the neprilysin and IDE [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 has proved that these pathways are activated through exercise [68]. On the other hand, cholinergic activity increases with exercise and cholinergic systems by physical activity involved in neuronal plasticity induced by exercise [66]. Mentioned is probably exercise can improve behavioral disorders by reducing the number of peptide A β 42 by increasing the production of neurotrophic factors (NGF, BDNF, and IGF-1) which are important for neuronal survival, a proliferation of neuronal and synaptic plasticity [69]. Also, physical activity reduces A β plaque and improves spatial learning (three-dimensional), memory, synaptic plasticity, and nerve tissue of Alzheimer's disease rat [70]. Several studies have reported that the cytoplasmic surface of apoptotic markers such as Cytochrome C, caspases-9, caspases-3, and Bax protein was significantly decreased in the brains of active Alzheimer's rat to the inactive Alzheimer's rat. In Alzheimer's rats, 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 A β [65, 71]. However, Park et al (2010) has provided a cyclic process stimulate A β in inflammation and suggested that signaling TNF- α , ultimately leads to the production of A β peptides causing produce new pathogenic A β peptides and increase its production and thus leads to Alzheimer's disease that can create a stronger cycle [72]. In this regard, Nichol et al (2008) demonstrated that inflammatory markers (IL-1 β and TNF- α) in the hippocampus of Alzheimer's transgenic rat was higher than the healthy rat and the level of anti-inflammatory agents (IFN- γ and MIP-1 α) in Alzheimer's transgenic rat was lower than the healthy rat. After 3 weeks of training, the levels of IL-1 β and TNF- α decreased and was close to the normal group that this reduction was associated with an increase in IFN- γ and MIP-1 α . Also a significant decrease in the levels of A β 40 solution and fibrillar A β solution in the cortex of Alzheimer's transgenic rat by 3 weeks optional practice was observed [73]. Also, Um et al (2008) showed that the SOD-1 protein and catalase in the brains of active Alzheimer's rat has a significant increase compared to the inactive. 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 A β 42 clinical reduced in rat with Alzheimer's disease [65]. It has been shown that 16 weeks of treadmill exercise combined with α -lipoic acid, decreased levels of brain A β 42 transgenic Alzheimer's rat. 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 increased the production of A β . Exercise alone and in combination with α -lipoic acid supplementation resulted in increased levels of oxidative stress and antioxidants as immunosuppressive agents and finally resulting in reduced apoptotic index and A β [71]. Possible mechanisms in this regard include the reduction of oxidative stress and increase antioxidant defense enzyme activity act as an agent enhancing the α -secretase and inhibitor β - and γ - secretase and so the processing of APP to conduct the Non-amyloidogenic pathway [74]. The improvement in A β 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 Alzheimer's disease, but generally, each has serious side effects. Thus, lifestyle changes such as exercise and nutrition can be used as a method to complement 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, focused to inhibit γ -secretase and reduction of A β 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 A β hippocampus of Alzheimer's disease subjects. In total, aerobic exercise training and omega-3 intake can be studied as complementary therapy in Alzheimer's patients.

Declarations

Ethics approval and consent to participate: all procedures consisted of a cannula, inducing AD inducing, the training protocol and killing and biopsy were done according to the regulations of the biological research ethics committee of Mazandaran University, and my manuscript does not contain any individual person's data thus not applicable.

Availability of Data and Materials: Please contact author for data requests.

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Figures

Figure 1

Aβ42 levels in the hippocampus of groups. * Significant difference than AC group in P<0.05.

Figure 2

γ - secretase levels in the hippocampus of research groups. * Significant difference than AC group and & significant difference than AT group in $P \leq 0.05$.

Figure 3

Neprilysin levels in the hippocampus of research groups.