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The Effect of Six Weeks-Voluntary Wheel Running on Brain Amyloid Beta (1-42) Levels of Diabetic Rats

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Abstract

***Background*:** Amyloid Beta (1-42) is derived from amyloid precursor protein and plays a critical role in AD pathogenesis. The purpose of this study was to investigate the effect of 6 weeks of voluntary wheel running on brain Amyloid beta (1-42) in the diabetic rats induced with alloxan.

***Materials and Methods*:** 28 male rats weight 185±1 were assigned randomly to 4 groups (N=7): normal control (C), training (T), control-diabetic (CD) and diabetic-training (DT). Diabetes was induced with injecting Alloxan (120 mg/kg dissolved in saline) intraperitoneal.

***Results*:** 6 weeks of voluntary wheel running decreased the cortex $A\beta_{1-42}$ in T and DT groups. $A\beta_{1-42}$ levels significantly decreased in the T and DT in compare with C and CD ($p<0.001$), respectively. Also $A\beta_{1-42}$ levels significantly increased in the CD in compare with C ($p<0.001$).

***Conclusion*:** voluntary exercise had positive effects on decreasing of $A\beta_{1-42}$ levels during 6 weeks. Therefore it can be recommended as therapeutic strategy for diabetes.

Introduction

Amyloid beta protein (Aβ) is one of the main factors in Alzheimer's pathogenesis [1, 2]. Accumulation and production of Aβ, are known as mechanisms to stimulate Alzheimer's disease [3, 4]. Aβ is formed through proteolysis of successive amyloid precursor protein (APP) [5]. The most common form of Aβ, is Aβ1-40 which is naturally secreted from the cells and It seems to be physiologically active [6]. Other species of Aβ, is Aβ1-42, which is more prone to accumulate. During life, Aβ1-40 (90%) and Aβ1-42 (10%) are naturally produced in the brains of healthy people [7].

Accumulation of Aβ1-42 results in amyloid plaque formation, and begin the cascade of events associated with neuronal and neurological disorders, inflammatory response, increased phosphorylation of protein tau, nerve cell death, neurological transporters disorder and clinical dementia [8, 9]. This protein, has the ability to generate free radicals from hydrogen peroxide as well as stimulating inflammatory cells [10]. Aβ1-42 protein in astrocytes and in neurons can cause Reactive Oxygen Species (ROS) production through NADPH oxidase [11, 12]. Diabetes is involved in the brain amyloid and tau metabolism [13].

Changes in insulin and glucose homeostasis of peripheral organs of the body may influence brain insulin and its receptor function, and will increase amyloid beta oligomerization and tau hyperphosphorylation [13, 14]. Beta-amyloid changes have been reported in diabetic human and animal subjects, and some researches have been suggested an increase in amyloid of the brain in diabetic subjects. However, the amounts of Aβ in the brain of 70_2 cadavers have not been affected by excessive blood sugar [15]. Janson et al. have examined 92 human samples (male and female) and reported no increase in the brain Aβ of 28 diabetic patients [16]. Other study showed no changes in Aβ levels (Aβ1-40, Aβ1-42) of cortex and hippocampus of diabetic rats (induced by Streptozotocin injection) after 10 days of hypo-insulinemia [17]. Another study found no changes in the brain Aβ levels of diabetics who underwent high-fat diet [18]. However, the data indicate that insulin resistance caused by diet, increases Aβ41 and Aβ1-42 production in transegenic Alzheimer's mice [19].

Li et al. showed that Aβ1-42 levels in the brain (frontal cortex) of type 1 and 2 diabetic rats have been increased significantly [20]. Jolivalt et al., indicated that 9 weeks of diabetes increased brain Aβ levels (whole brain except cerebellum) in animal subjects [21]. Another study showed that the levels of Aβ1-40 and Aβ1-42 increased in the brains of Alzheimer's female rats with high-fat diet and after 8, 12 and 16 weeks of age [22].

Running wheel will exert significant effects on rodent brain and behavior. Running wheel is a form of voluntary activity that is used in research on animal models and is a useful tool for neurobehavioral study in rodents. Unlike the harmful effects of forced treadmill running exercise on cognitive ability, voluntary training will not impose chronic stress associated with forced exercise. Several studies have reported contradictory results on the effects

of exercise and physical activity on levels of brain Aβ. In this regard, a study showed that 3-week running wheel reduced soluble Aβ fibrylar in rat cortex with Alzheimer's disease. However, differences between cortex soluble and insoluble Aβ42 of sedentary and active Alzheimer's rats have not been found to be statistically significant. Exercise also reduces Aβ fibrillary and improves behavior in rats with Alzheimer's disease. Aβ damage and cognitive decline in Alzheimer's disease can be improved through stimulation of the adaptive immune response [23].

Another study showed that 3-week voluntary running wheel does not change levels of insoluble amyloid beta (Aβ1-40, Aβ1-42) in the brain (hippocampus) of Alzheimer's rats [24]. In addition, 4 months of forced and voluntary running had no significant effects on levels of soluble Aβ (Aβ1-40, Aβ1-42) in the cortex and hippocampus of Alzheimer's rats [25]. However, followed by 5 months of voluntary running wheel significant reduction was observed in the extracellular Aβ plaques in the frontal cortex and hippocampus and cortex of Alzheimer's rats [26]. Running on a treadmill for 16 weeks significantly reduced the brain Aβ1-42 protein levels in Alzheimer's and non Alzheimer's rats [27]. In another study, 5 weeks of treadmill running reduced levels of Aβ in Alzheimer's rats brain [28].

Consequently, there is no consensus in the literature regarding the effects of exercise on amyloid beta. Some of them showed that the exercise reduces this protein but others have reported no change. Given these conflicting results and lack of information about the effect of voluntary exercise on amyloid beta levels in diabetic subjects, this study investigated the effects of 6 weeks voluntary training on the amyloid beta protein levels in cortex of diabetic rats.

Materials and Methods

In this study, 28 adult male Wistar rats (8 weeks of age and weight of 185±1 g) were randomly divided into 4 groups (7 rats in each one): 1- the healthy control group (C): this group was not diabetic and did not exercise, 2- Training (T): this group was kept in cages equipped with wheels for 6 weeks, 3- Diabetic control (CD): the diabetic group was kept in cages without performing exercise for 6 weeks, and 4- Diabetic Training (DT): the diabetic group was kept in cages equipped with running wheels for 6 weeks.

The maintenance and care of the experimental rats were in accordance with the guidelines of the Helsinki convention. Subjects were kept in standard condition (temperature of 22±2ºC with relative humidity of 50% and the 12:12 hours light/dark cycle) and had free access to food and water. After 16 hours of fasting, alloxan (120 mg/kg) was dissolved in saline and injected to rats intraperitoneally. Five days after alloxan injection, their blood glucose levels were measured by sampling blood from their tail [29, 30]. Those with higher blood glucose concentrations from 250 mg/dl were identified as diabetic [29].

Training groups (2 groups, total of 14 rats) were kept individually in cages equipped with wheels (manufactured by School of Physical Education, Mazandaran University) and had free access to the wheels. This machine is equipped with counter that shows the mileage during the day. Mileage provided by any of the subjects was recorded at an exact time each morning. Subjects were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (6 mg/kg). To collect samples of cortex, the subjects were decapitated by scissors, the skull has been split using surgical scalpel and the brain was removed. Normal cortex of the brain was isolated by a surgical scalpel. Brain tissue was frozen by liquid nitrogen at 80ºC. The homogenized brain tissue was centrifuged at the speed of 10,000 rpm for 5 minutes [25].

The supernatant was harvested and frozen by liquid nitrogen and were stored at 80ºC for subsequent measurements. Cortex levels of Aβ1-42 were measured with ELISA method according to manufacturer's instructions (Wuhan, China). Coefficient of variation (CV) and sensitivity of this method was estimated to be 6.3% and 0.24 pg/ml, respectively. After collecting data, and to compare variables of four groups, the Kolmogorov-Smirnov test was used for examining normality of the data and One-way ANOVA and LSD post hoc tests to examine differences between Aβ1-42 of each group. All calculations were performed using SPSS-19 statistical software, and significance domain was determined to be $p \leq 0.05$.

Results

Table 1 shows Weight values and cortical Aβ1-42 levels related to separate groups. The distance run by diabetic subjects was less than healthy subjects. The 6-week voluntary running exercise in healthy mice, decreased cortical Aβ1-42 levels significantly in group T ($p<0.001$). Also, the amount of cortical Aβ1-42 levels of DT group have been decreased compared to CD group ($p<0.001$). Cortical Aβ1-42 levels of CD group significantly increased compared to group C ($p<0.001$).

However, cortical Aβ1-42 levels were not significantly different from T group compared with DT ($p=0.411$). The data associated with cortical Aβ1-42 levels, weight and distance (m) in each group are presented in figure 1 and table 1, respectively.

Table 1. Values of cortical Aβ1-42 levels, weight and running distance in research groups

variable	Diabetes training (Mean±SD)	Control diabetes (Mean±SD)	Trining (Mean±SD)	Control (Mean±SD)
Aβ1-42	0.451±0.053	1.32±0.30	0.51±0.049	0.98±0.053
Weight	233.71±24.93	231±5.59	326.14±3.28	348.29±6.65
Running distance (m)	650.95±98.25	-	3244±385.57	-

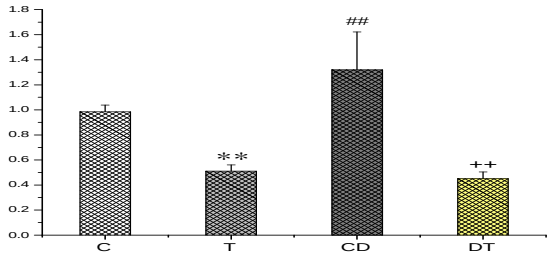

Figure 1. Aβ1-42 levels of subjects' brain cortex (**) Significant difference with control group (p<0.05); (++) significant difference with control group - diabetes (p<0.05); (##) significant difference with control group (p<0.05).

Discussion

In this study, weight and distance values in diabetic subjects were lower than in healthy subjects indicating the negative impact of diabetes on body weight and distance in the diabetic subjects. Cortical Aβ1-42 levels in CD group showed a significant increase. The injection of alloxan induced diabetes in CD and DT groups. Diabetes induction increased cortical Aβ1-42 levels. There were no significant differences in levels of Aβ1-42 in T and DT groups. Accumulation of amyloid beta has damaging effects on brain [31]. Accumulation of this protein as plaques in the brain is one of the main factors in Alzheimer's disease. Aβ accumulation in brain of diabetic rats has been associated with nerve inflammation and neuronal damage [20].

Several possible mechanisms are identified for the relationship between diabetes and Aβ. For example, insulin deficiency is the primary event that leads to decrease in insulin signaling cascade in brain and behavioral disorders caused by diabetes. Deficiency in insulin signaling is associated with diabetes mellitus and may be the result of insulin deficiency (Type 1) or insulin resistance (Type 2). Reduced insulin signaling is related to increase in levels of Aβ protein. In other words, the protein levels of Aβ increases significantly in the brain of diabetic rats. Insulin degradation enzyme (IDE) can breakdown Aβ [16, 32]. Insulin deficiency and reduced signaling may contribute to reduced IDE expression and increased Aβ protein levels [21, 33]. The findings of the present study is consistent with other studies that reported increase in levels of Aβ1-42 induced by diabetes. The present study showed that 6 weeks of voluntary exercise reduced Aβ1-42 levels in healthy and diabetic exercise groups. Adlard et al. showed that 5 months of voluntary wheel running leads to a significant decrease in extracellular Aβ plaques in the frontal cortex (38%) and cortex located in the hippocampus (53%) and hippocampus (40%). They stated that voluntary exercise may mediate the amyloid precursor protein metabolism and Aβ cascade resulting decrease in the production of Aβ [26]. Another possible mechanism in relation to exercise and Aβ levels is that up-regulation of Proteasome activity induced from exercise [34] can mediate proteolytic fragments degradation of amyloid precursor protein (which plays an important role in the production of Aβ) [35]. Cho et al. showed that 16 weeks of treadmill running decreased the Aβ1-42 levels in 13-months-old Alzheimer's rats. The results of their study showed that physical activity, possibly through the regulation of amyloid precursor protein or increasing amounts of degradation and scavenging Aβ plaques can reduce brain Aβ [36].

What is noteworthy in this study is that exercise could decrease Aβ1-42 protein levels of brain in diabetic subjects which indicates that voluntary exercise has positive effects in reducing the consequences of diabetes. The findings of this study showed that voluntary exercise can reduce Aβ load in an animal model of diabetes. While pharmacological interventions are seeking to reduce Aβ to block cascade of brain diseases especially Alzheimer's disease, the findings of the present study showed that voluntary exercise is a simple behavioral strategy that can induce resistance against the development of diabetic neuropathology in brain. According to this study it seems that voluntary exercise can reduce the accumulation of amyloid-induced diabetes in animal subjects, and hence can be effective as a nonpharmacological therapy.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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