

# The Effects of Exercise on Cerebellar Growth-Associated Protein 43 and Adenylyl Cyclase- Associated Protein 1 Gene Expression and Proteins in Diabetic-Induced Neuropathy and Healthy Male Wistar Rats

## Abstract

**Background:** The effect of exercise on the cerebellum cells in diabetic-induced neuropathy and healthy situations is not clear yet. Growth-associated protein 43 (GAP-43) and adenylyl cyclase-associated protein 1 (CAP-1) proteins can restore nerve cells. This study aimed to investigate the effect of aerobic exercise on GAP-43 and CAP-1 and their mRNA in the cerebellar tissue of diabetic-induced neuropathy and healthy Wistar rats. **Methods:** Around 40 healthy male Wistar rats with a mean weight of  $271 \pm 11.2$  g were divided randomly into four groups; healthy aerobic exercise, diabetic-aerobic exercise, healthy-control, and diabetic-control. The exercise group performed aerobic exercise 5 days per week for 6 weeks. **Results:** Exercise increased CAP1 protein in the cerebellum tissue of healthy ( $P = 0.002$ ) and diabetic ( $P = 0.002$ ) groups compared with matched control groups. The effect of exercise on CAP1 was greater in diabetic compared with the healthy group ( $P = 0.002$ ). The expression of CAP1 mRNA in the cerebellum was higher in the healthy exercise compared with the healthy control group ( $P = 0.002$ ) and in the healthy exercise compared with the diabetic exercise group ( $P = 0.026$ ). GAP43 protein was lower in the healthy exercise compared with the healthy control group ( $P = 0.002$ ) while it was higher in diabetic exercise compared to the healthy exercise group ( $P = 0.002$ ). Expression of GAP43 mRNA in the cerebellum was higher in the healthy ( $P = 0.002$ ) and diabetic ( $P = 0.002$ ) exercise groups compared to non-exercise matched groups and in the diabetic control group compared with the healthy control group ( $P = 0.002$ ). Exercise improved latency in diabetic ( $P = 0.001$ ) and healthy exercise groups ( $P = 0.02$ ). No significant difference was found in blood glucose between exercise and control groups ( $P > 0.05$ ). **Conclusion:** Exercise improved cerebellar functions in healthy and diabetic rats, probably mediating by CAP1 protein, even without changing blood glucose.

**Keywords:** Aerobic exercise, cerebellum, diabetic neuropathies

## Introduction

The International Diabetes Federation reported that 382 million people in the world are currently affected by diabetes. The disease usually progresses to involve various organs and causes several diseases including peripheral<sup>[1,2]</sup> and central neuropathy.<sup>[3]</sup> Diabetic neuropathy patients often experience various abnormal sensations including hypersensitivity to mechanical or thermal stimuli (namely hyperalgesia and allodynia) followed by the long-term contradictory loss of stimulus-evoked sensation.<sup>[4]</sup> Cerebellar damage impairs internal predictions for sensory and motor function.<sup>[5]</sup> Cerebellum as a part of the central nervous system which has important roles in motor control,

reflex adaptation, and motor learning can be affected by diabetes,<sup>[6,7]</sup> and ultra-structural changes are found in the cerebellum of diabetic rats.<sup>[8]</sup> Structural plasticity of the nervous system including the cerebellum can occur after brain damage to reconstruct appropriate synaptic connections. This capacity depends on several mediating factors including growth-associated protein 43 (GAP-43) and adenylyl cyclase-associated protein 1 (CAP1).

GAP-43 is a novel axonal phosphoprotein, and its expression is associated with increased synaptic plasticity in the brains *in vivo*<sup>[9]</sup> and is essential for promoting denervation-induced sprouting and maintaining normal nervous tissue structure.<sup>[10,11]</sup> The growth cone plays crucial roles in neural wiring, synapse formation,

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and axonal regeneration.<sup>[12]</sup> GAP43 is a major growth cone protein that controls F-actin dynamics, and it is used as a marker for developing neural regeneration.<sup>[13]</sup> There are controversies regarding the effects of diabetes on GAP43 in the neural system. Diabetes mellitus can downregulate GAP43 in the hippocampal area<sup>[14]</sup> or increase GAP43 in the skin nerve fibers of patients with type 2 diabetes mellitus.<sup>[15]</sup>

CAP1 is another protein that is probably related to growth cone. It is a member of cyclase-associated proteins involved in the regulation of actin filaments. CAP1 protein expression was associated with astrocyte proliferation after the trauma of the central nervous system (CNS).<sup>[16]</sup> CAP1 can regulate growth cone morphology through rearrangement of F-actin and so regulating neurite growth.<sup>[17]</sup> CAP1 role has also been identified as resistin receptor<sup>[18]</sup> and regarding the association of resistin with insulin resistance,<sup>[19]</sup> CAP1 may also mediate neural regeneration. Several factors including exercise can enhance neural regeneration. Various studies have indicated the positive influence of exercise on nerve function or regeneration in diabetes<sup>[20,21]</sup> while studies related to the effects of exercise on the cerebellum are limited. It has been indicated that aerobic exercise increases the number of synapses per Purkinje cell (the large, primary nerve cell in the cerebellum).<sup>[22]</sup> Regular exercise could improve blood diffusion and angiogenesis of the cerebellum in adult rats<sup>[23]</sup> and it was hypothesized that increasing blood diffusion improves neural regeneration and related factors as CAP1. However, the effects of exercise on CAP1 as a regulator of neurons in the cerebellum in diabetics or healthy subjects are not clear yet.

The physiological mechanism related to exercise-induced synaptic plasticity could be the involvement of several molecules associated with the maintenance and regulation of brain function, such as neurotrophic factors, signal transduction proteins, transcription factors, and synaptic proteins.<sup>[24,25]</sup> Exercise is also known to enhance the expression of neurotrophic factors, which could increase the GAP-43 expression and the nerve regeneration capacity.<sup>[26-28]</sup> Although the exercise was predicted to reduce cerebellar neuropathy by reducing inflammation and blood glucose,<sup>[29]</sup> GAP-43 and CAP1 as cellular targets and their roles especially in diabetes and related to exercise training, need to be clarified yet. On the other hand, in many available studies, gene expression as mRNA has been used as an indicator of the effect of exercise while RNA-level regulation appeared to be stronger than that of protein-level changes, and the relative importance of transcription, translation, and degradation for some genes in various situations may be varied.<sup>[30]</sup> In other words, mRNA may not necessarily predict its related proteins. Generally, first, there are no clear findings regarding the effect of exercise on the cerebellum structure and function in healthy and diabetic subjects; secondly, the role of two new proteins of CAP1 and GAP43 regarding the effect of exercise on

cerebellum is not clear; and thirdly, it is not clear whether, in healthy and diabetic situations, CAP1 and GAP43 gene expression (mRNA) are associated with the translation of proteins. Therefore, the present study aimed to evaluate the influence of aerobic training on GAP43 and CAP1 proteins and related mRNA in STZ-induced diabetic and nondiabetic rats.

## Methods

### Animals and treatment

Around 40 male Wistar rats with the initial weight of  $271 \pm 11.2$  g and the average age of 10 weeks were housed in a count of three in Plexiglas cages with latticework doors in  $25 * 27*43$  dimensions at a constant temperature of  $22 \pm 2^{\circ}\text{C}$  with a 12-h light-dark cycle. Rats had *ad libitum* access to rat pellet (protein = 26%, carbohydrate = 60%, and fat + 14%) and tap water. After 2 weeks of adaptation to static treadmill and laboratory environment, the rats were randomly divided to 4 groups: healthy control group (HC) which was without diabetes or exercised, diabetic control group (DC) in which diabetes was induced but did not perform exercise training, healthy exercise group (HE) in which diabetes was not induced but they performed exercise training, and diabetic exercise group (DE) in which diabetes was induced and performed exercise training. The study proposal and procedures were approved by the Institutional Ethics Committee of Animal Studies at Shiraz University and followed the ethical guidelines for the care and use of animal labs, published by the National Institute of Health.

### Diabetes induction

Following a night fasting, diabetes was induced in rats with an intraperitoneal injection of 45 mg/kg streptozotocin (STZ) (Sigma-Aldrich, St. Louis, MO; citrate buffer 0.1 M, pH 4.5) which could mimic type 1 diabetes.<sup>[31]</sup> Blood glucose was taken from tail vein blood using Acu-Check Go One Touch glucometer (Roche Diagnostics) 6 h following fasting and 48 h after induction of diabetes at the initial stage of study and 24 h after the last bout of exercise. The diabetic state was confirmed when the non-fasting blood glucose level was higher than 300 mg/dL.<sup>[32]</sup> The control group received citrate buffer instead of STZ.

### Behavioral test of neuropathy

Rats' neuropathy was confirmed in the case of observing early symptoms of neuropathy including hyperalgesia and allodynia<sup>[33]</sup> or known as thermal and mechanical tests which assess behavioral measures of diabetic neuropathy. In hyperalgesia test threshold to radiant heat was assessed using the hot-plate paw withdrawal test. A 40-cm-high Plexiglas cylinder was suspended over the hot plate, and the temperature was kept at  $50^{\circ}\text{C}$  to give a latency period of approximately 10 s for control rats. Withdrawal

latency was the time between placing the rat on the hot plate and the time of withdrawal of the hind paw. For the mechanical allodynia test, the rats were qualified by a dynamic paw withdrawal test with a Dynamic Plantar Aesthesiometer (Ugo Basile, Comerio, Italy), which generates a progressive mechanical force. The paw withdrawal reflex was recorded by recording the latency until withdrawal in response to the mechanical force.

About 2 weeks after STZ infusion, rats with neuropathic symptoms were regarded as diabetic neuropathy model and other rats were excluded from the procedure. During every 2 weeks of the study, neuropathy tests were performed.

### Body weight measurement and training program

The bodyweight of all animals was measured 6 h following fasting during the first session and 24 h following the last session of the experiment at 14–15 pm. About 5 days before the main exercise training program, exercise groups were habituated with treadmill running at the speed of 5 m/min for 1–5 min (grade 0%). The exercise training protocol was designed according to the “Resource Book for the Design of Animal Exercise Protocols.”<sup>[34]</sup> Moderate-intensity treadmill training was performed 5 days per week for 6 weeks. The rats ran on the treadmill with an incremental increase of speed and duration from “10 m/min for 10 min (grade 0%) in the 1<sup>st</sup> week to 10 m/min for 20 min (grade 0%) in the 2<sup>nd</sup> week, 14–15 m/min for 20 min (grade 0%) in the 3<sup>rd</sup> week, 14–15 m/min for 30 min (grade 0%) in the 4<sup>th</sup> week, and 17–18 m/min for 30 min (grade 0%) for the 5<sup>th</sup> and 6<sup>th</sup> week. The non-exercise groups were kept on the static treadmill during the same time and duration as exercise groups to be controlled the environmental effects.

### Tissue preparation

Nearly 24 hours after the last session of the exercise program, the rats were anesthetized with an intraperitoneal injection of combined xylazine (5 mg/kg) and ketamine (75 mg/kg). A part of the cerebellum was stored at nitrogen for real-time PCR and another part was fixed in formalin 10% for immunoassays. For light microscopic analysis, cerebella were cryoprotected with 30% sucrose in PB and were cut in the sagittal section at postnatal day 5 at a thickness of 20  $\mu$ m by a cryostat. The sections were prepared in gelatin-coated glass slides.

### Immunohistochemistry assay

For immunohistochemistry, the separated sections were washed in 0.01 M PBS containing 3% Triton X-100 (pH 7.4, PBS-T), then inserted in X10 normal goat serum in PBS for 45 min at 37°C, and then incubated at 4°C with monoclonal anti-GAP-43 (1:200, Abcam) or anti-MKP-1 (1:200, Monoclonal MKP-1 antibody, Santa Cruz Biotechnology, Lot# D0904, USA) in PBS for overnight, washed in PBS (3  $\times$  5 min), incubated in biotinylated horse anti-mouse IgG (1:200, Boster) in PBS

for 2 h at room temperature, washed in PBS (3  $\times$  5 min), incubated in avidin-biotin-peroxidase complex solution (ABC, 1:100, Boster) for 2 h, then washed again in PBS (3  $\times$  5 min). The nucleus stained by incubating the tissue by DAPI for 15 min.

### Analysis of gene expression by Real-Time PCR

For analysis of mRNA levels, total RNA was isolated using a guanidine/phenol solution (Qiazol lysis reagent -USA) according to the manufacturer’s protocol. RNA quantity and quality were assessed by NanoDrop 2000 (Thermo scientific) instrument. Then, 1  $\mu$ g of total RNA was taken for the first-strand complementary DNA (cDNA) synthesis reaction (Thermo Scientific RevertAid First Strand cDNA Synthesis Kit) according to the manufacturer’s protocol.

The relative expression of the messenger RNA (mRNA) was analyzed by preparing reaction mixer with PCR Master Mix (2X) (Korea-Biofact) and gene-specific primers with diluted cDNA and final volume made up to 10  $\mu$ L with nuclease-free water. Fluorescently tagged probes and forward and reverse PCR primers, designed by Integrated DNA Technologies (Pishgam), consisted of the following: 5’GGAATAAGGATCCGAGGAGGAAAGGAG3’, 5’CTTAAAGTTCAGGCATGTTCTTGGT3’ for rat GAP-43 GAP43,

Probe 5-CTCATAAGGCTGCAACCAAAATTCAGGCT-3,

forward primer 5-GATGGTGTCAAACCGGAGGAT-3,

reverse primer 5-CTTGTTATGTGTCCACGGAAGC-3.

Quantification and analysis were carried out in ABI real-time PCR. To determine the relative levels of GAP43 and CAP1 mRNAs Quantitative real-time RT-PCR was used. The target gene expression was normalized to the housekeeping gene GAPDH, and relative expression was determined using Delta Delta Ct (DDCt) method.

### Statistical analysis

The statistical analyses were performed using SPSS version 19 software. Regarding the non-normality of data distribution approved by the Shapiro-Wilk test, the nonparametric test of Kruskal-Wallis was used for comparing groups and the Mann-Whitney test was used for comparing paired groups. For the comparisons, paired groups were considered healthy control rats versus healthy exercise rats, healthy control rats versus diabetic control rats, healthy exercise rats versus diabetic exercise rats, and diabetic exercise rats versus diabetic control rats.

### Results

Compared to healthy rats, in diabetic groups (exercise and non-exercise) blood glucose during the 3<sup>rd</sup> and 6<sup>th</sup> weeks of the experiment increased significantly ( $P = 0.001$ ). Hyperglycemia which was initiated by 400–450 mg/dL remained elevated in diabetic groups until the end of the



study. Exercise did not cause any significant effect on the blood glucose of diabetics and healthy rats ( $P > 0.05$ ). Weight decreased in diabetic groups following the 3<sup>rd</sup> and 6<sup>th</sup> weeks of the study compared to initial weight and healthy groups ( $P = 0.001$ ). Exercise did not cause a significant change in the weight of healthy and diabetic rats compared to matched non-exercise groups.

The latency of the paw withdrawal test in hyperalgesia heat and the changes in the paw withdrawal threshold in the mechanical allodynia test was reduced in exercise and non-exercise diabetic rats compared with healthy rats. Following the 3<sup>rd</sup> and 6<sup>th</sup> weeks of experiment mechanical and thermal measures increased in healthy and diabetic exercise groups compared with matched non-exercise groups ( $P < 0.05$ ). Paired group comparisons using Mann-Whitney test [Table 1] indicated that following 6 weeks of the experiment:

CAP1 protein in cerebellum increased in healthy ( $P = 0.002$ ) and diabetic ( $P = 0.004$ ) exercise groups compared with matched control groups and also in the diabetic exercise group compared with the control exercise group ( $P = 0.023$ ). However, there was no significant difference in CAP1 of healthy and diabetic control groups ( $P > 0.05$ ) [Figure 1 and Table 1].

The expression of CAP1 mRNA in the cerebellum was increased in the healthy exercise compared to the healthy control group ( $P = 0.002$ ) and in healthy exercise compared to the diabetic exercise group ( $P = 0.002$ ). There was no significant difference in CAP1 mRNA of other paired comparisons ( $P > 0.05$ ) [Figure 1 and Table 1]. GAP43 protein in the cerebellum was significantly lower in healthy exercise compared to healthy control (0.004) and diabetic exercise ( $P = 0.004$ ) groups [Figure 2]. There was no significant difference in GAP43 between other groups ( $P > 0.05$ ) [Figure 2 and Table 1].

Expression of GAP43 mRNA in the cerebellum was higher in healthy exercise ( $P = 0.002$ ) and diabetic exercise ( $P = 0.004$ ) groups compared to non-exercise matched groups and in the diabetic control group compared to the healthy control group ( $P = 0.002$ ). There was no significant difference in GAP43 of exercise diabetic vs exercise control groups ( $P = 0.05$ ) [Figure 2 and Table 1].

## Discussion

Findings of the present study indicated that diabetes caused hyperglycemia which was sustained in diabetic exercise and non-exercise groups until the end of the

experiment. Exercise did not cause any significant effect on blood glucose in healthy and diabetic rats. Besides, diabetes reduced the weight of rats but the exercise was not effective on weight. Although the exercise program did not affect blood glucose, it was effective in diabetic neuropathy which was tested by mechanical and thermal measures. Neural functions were reduced in exercise diabetic rats compared with matched healthy rats following the 3<sup>rd</sup> and 6<sup>th</sup> weeks of exercise. Even in healthy rats, exercise improved mechanical and thermal measures of neural functions. This means that even in the situations that exercise program is not effective on hyperglycemia or in healthy subjects, aerobic exercise can improve neural functions. Diabetic neuropathy can occur as a result of hyperglycemia which damages nerve cells and cause neural ischemia.<sup>[35]</sup> Besides, an increasing flux of the polyol pathway, increased advanced glycation end-product formation, excessive release of cytokines, activation of protein kinase C and enhancing oxidative stress, and other factors could cause nerve injury or neuropathy.<sup>[36]</sup> Exercise could increase vessel blood flow or have a protective role for nerve injury through increasing antioxidant capacity and reducing glycated end and many other nerve injury factors.<sup>[37]</sup> Thus, exercise could reduce neuropathy without changing blood glucose.

The physiological mechanism of exercise effect on the improvement of neural functions includes several other molecules that regulate brain function, such as neurotrophic factors, signal transduction proteins, transcription factors, and synaptic proteins.<sup>[24,25]</sup> However, cellular mechanisms of exercise-induced neural regeneration or function especially in diabetes situations are not completely clear yet.

CAP1 and GAP43 have been offered as two mediating factors in neural regeneration.<sup>[11]</sup> Our findings indicated that firstly, diabetes without exercise did not change CAP1 which may be related to the small number of samples or short duration of neuropathy for inducing significant cerebellar degeneration; secondly, exercise increased CAP1 protein in cerebellar tissue of healthy and diabetic groups compared with matched control groups; and thirdly, the effect of exercise on CAP1 was greater in diabetic compared with the healthy group. We can conclude that CAP1 is probably a mediating factor for exercise effect in improving diabetic neuropathy and in the situation of diabetes in which neural degeneration could be more prominent, exercise could increase CAP1 in diabetic more than healthy subjects as a compensation mechanism.

**Table 1: Mean±SD of CAP1 and GAP43 proteins and mRNA in four groups**

Groups	CAP1 (µm <sup>2</sup> ) * $P=0.026$	CAP1 mRNA (ratio)	GAP43 (µm <sup>2</sup> )	GAP43 Mrna
Healthy-control	1911.2±354.85	0.98±0.01	14621±2559	0.96±0.01
Healthy-exercise	15437±3076.71	1.26±5.77	6400.50±2708.40	2.26±0.65
Diabetic-control	6898.9±680.43	1.03±0.18	16295±9230.92	1.76±0.36
Diabetic-exercise	19811±2489.85	0.92±0.1	22821±2946.68	4.11±1.16

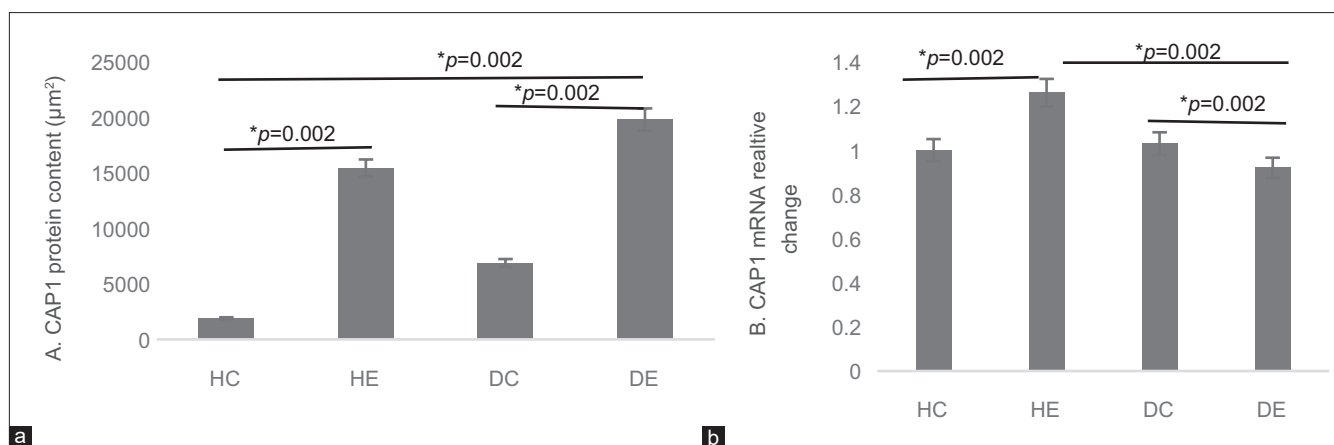


Figure 1: (a) CAP1 protein content and (b) CAP1 mRNA of four groups of study (HC: healthy control, HE: healthy exercise, DC: diabetic control, DE: diabetic exercise). \* : Significant difference ( $P < 0.05$ )

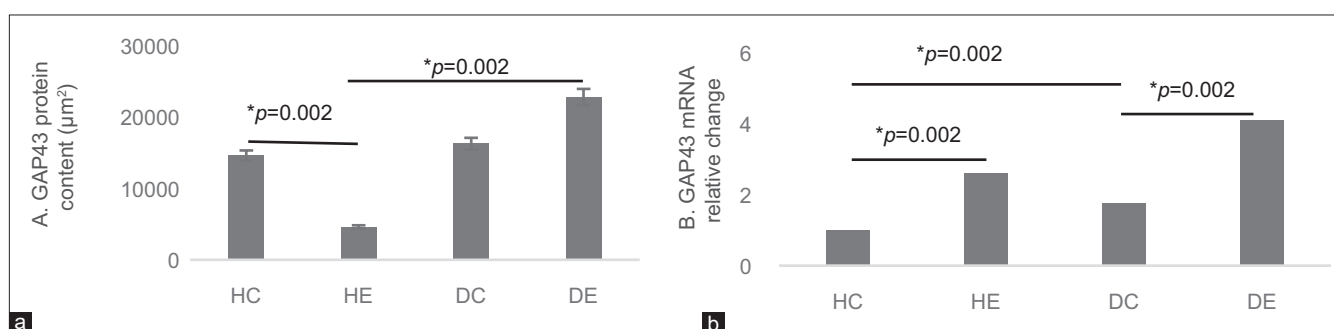


Figure 2: (a) GAP43 protein content and (b) GAP43 mRNA of four groups of study (HC: healthy control, HE: healthy exercise, DC: diabetic control, DE: diabetic exercise). \* Significant difference ( $P < 0.05$ )

Zhang *et al.*<sup>[16]</sup> indicated the role of CAP1 in neural regeneration and found that the change of CAP1 protein expression was related to astrocyte proliferation after the trauma of the central nervous system (CNS). Recently, Rahmati and Kazemi<sup>[38]</sup> found that mild exercise intensity did not change CAP-1 expression which contradicted our findings while high-exercise intensity (HEI) increased CAP-1 expression in the hippocampus of rats which was consistent with our findings. The difference of the present study may be related to exercise intensity of our study which was higher than the mild and lower than the high intensities of exercises in the mentioned studies as well as the difference in the CNS tissues (cerebellum not hippocampus). In the findings of Rahmati and Kazemi,<sup>[38]</sup> the induction of corticosterone release associated with high-intensity exercise has been mentioned as an effective factor in the hippocampal CAP-1 expression levels. CAP1 can improve nerve growth cone morphology through rearrangement of F-actin and regulate neurite growth,<sup>[17]</sup> and probably because of degeneration of growth cone in diabetic subjects compared to healthy ones, exercise could produce greater CAP1 in diabetic subjects.

For evaluating the origin of CAP1 protein changes as a result of exercise and diabetes, we considered gene expression of CAP1 mRNA. Our findings indicated that

exercise increased expression of CAP1 mRNA in the cerebellum of the healthy exercise compared to the healthy control group which was interestingly different from CAP1 protein, CAP1 mRNA expression was greater in the healthy exercise compared to the diabetic exercise group. Thus, we can conclude that firstly, CAP1 protein changes are not necessarily matched with its gene expression and secondly, exercise and diabetes may be effective on mediators of protein translation process differently. At various levels of protein translation, several factors could affect mRNA degradation, protein degradation, or ribosomal synthesis<sup>[38]</sup> and as a result, could cause CAP1 mRNA expression which was not comparable to CAP1 protein concentration.

Another protein which has been discussed as effective protein in neural regeneration is GAP43.<sup>[10,11]</sup> GAP-43 protein specifically binds to growth cones that regulate the most important neuronal functions, including synaptic plasticity<sup>[39]</sup> and axonal regeneration.<sup>[40]</sup> According to our findings, exercise reduced GAP43 protein in the cerebellum of the healthy exercise compared to the healthy control group, however, GAP43 protein increased in diabetic exercise compared to the healthy exercise group. It means that exercise reduced GAP43 in healthy subjects while increased it in diabetic subjects. Consistent with our findings in the healthy group, Rahmati, and

Kazemi<sup>[38]</sup> observed that HEI with the highest level of corticosterone and lactate production reduced GAP-43 expression in the hippocampus. This finding was not consistent with some of the other findings which indicated that mild exercise wheel running enhanced hippocampal GAP-43 expression in IGF-I-dependent manner,<sup>[41]</sup> or increased GAP-43 in the spinal cord,<sup>[28]</sup> hippocampus,<sup>[42]</sup> and cortex.<sup>[43]</sup> However, in none of the previous studies, the cerebellum was the target tissue and the exercise programs and the health status of subjects were different. Although considering many studies, enhancement of GAP-43 mRNA and/or protein activity has been hypothesized as a central mediator of axonal neuroplastic and regenerative responses the central nervous system, it does not appear to be the crucial substrate sufficient for driving these responses,<sup>[44]</sup> and in the present study, increasing of CAP1 in healthy subjects may be a compensatory mechanism for decreasing of GAP43. Furthermore, we evaluated the expression of GAP43 mRNA and found that expression of GAP43 mRNA expression was higher in the healthy exercise and diabetic exercise groups compared to the non-exercise matched groups and in the diabetic control group compared to the healthy control group. We concluded that exercise could increase GAP43 mRNA in both healthy and diabetic rats and this increase was greater in the diabetic compared to the healthy group. Diabetes also increased GAP43 mRNA in the non-exercise group.

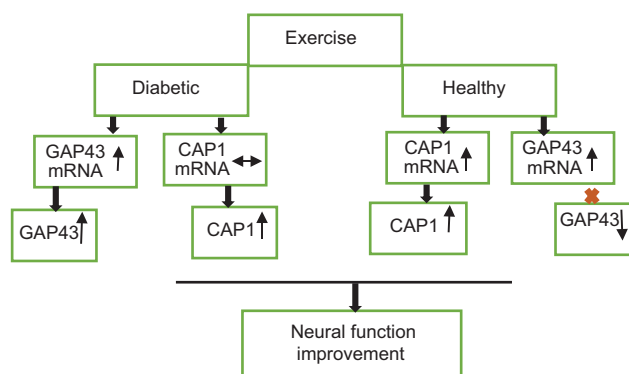
Our findings regarding the effect of exercise on GAP43 mRNA in healthy group was partly contrary to its related protein, which may indicate that in healthy subjects mediating factors such as the delay between transcription and translation which can regulate presence or removal of unnecessary proteins can be changed in various situations (such as diabetes or exercise) and is known as translation on demand.<sup>[45]</sup> Protein translation can be regulated by transcription factors that destroy or block the production of unnecessary proteins<sup>[46]</sup> which could suppress translation of GAP43 protein. Even, protein biosynthesis can be limited by limitation in energy nutrients, number and distribution of ribosomes, and several other factors. Probably in diabetics, mentioned factors were different from non-diabetics.

### Recommendations

Aerobic exercise can be recommended to improve neural function in healthy and diabetic situations while related mechanisms of genes and proteins and the effect exercise on antioxidants and other mediatory factors in cellular functions need to be clarified.

### Limitations

The small number of subjects, not assessing several brain regions, and other neural regeneration factors restrict the generalization of the findings and were the main limitations of the study.



**Figure 3: Figure Cap1 and GAP43 proteins and mRNAs interaction with exercise in healthy and diabetic rats. increase; decrease; no significant change**

### Conclusions

A summary and interaction of exercise and GAP43 and CAP1 in healthy and diabetic rats has been presented in Figure 3. This was the first study that assessed simultaneously CPA1 and GAP43 genes and proteins in the cerebellum and indicated a more prominent increase of regeneration factors as CAP1 and GAP43 in diabetics compared to healthy subjects, and just increasing of GAP43 in healthy subjects. Probably, GAP43 protein is not an important factor for neural regeneration stimulated by exercise in healthy subjects, and other regeneration factors such as CAP1 may be more important in neural function and mediating factors for GAP43 protein translation in healthy and diabetes situation may be different which needs future clarifications; GAP43 and CAP1 proteins and genes indicated some distinct behavior which can be associated with differences in regulatory protein synthesis and turnover, the required time for GAP43 and CAP1 gene expression, and protein translation as a result of exercise or diabetes; exercise could improve cerebellar neural function and regeneration marker even without changing blood glucose.

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### Conflicts of interest

There are no conflicts of interest.

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