Golestan University of Medical Sciences

Jorjani Biomedicine Journal

Online ISSN: 2645-3509

Examination of ATG5, ATG7, and Beclin1 gene expression in the soleus muscle of animal model Parkinson following eight weeks of endurance training with branched-chain amino acids supplementation

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Abstract

Background: Autophagy is associated with the degradation of intracellular organelles and, as a regulator of cellular homeostasis, can affect muscle atrophy. Therefore, this study aimed to examine ATG5, ATG7, and Beclin1 gene expression in the soleus muscle of an animal model of Parkinson following eight weeks of endurance training with branched-chain amino acids (BCAA) supplementation.

Methods: In this study, 25 male Wistar rats (weight = 180-200 g and age = 12 weeks) were used. First, the rats were divided into five groups (n = 5): healthy control, Parkinson control, Parkinson + training, Parkinson + BCAA, and Parkinson + training + BCAA. Eight weeks of moderate-intensity endurance training were performed. After anesthetizing the rats, the soleus muscles were isolated; then, RNA extraction, cDNA synthesis, and real-time PCR were used for gene expression analysis. One-way analysis of variance with Tukey's post-hoc test was used to analyze the data ($P \leq 0.05$).

Results: ATG5, ATG7, and Beclin1 gene expression in the soleus muscle of rats significantly increased after Parkinson's induction (P-Value = 0.001). After both interventions, there was a significant decrease in the gene expression of ATG5, ATG7, and Beclin1 (P-Value = 0.001), observed only in the endurance training with BCAA supplementation group.

Conclusion: Although endurance training and BCAA supplementation alone did not significantly affect ATG5, ATG7, and Beclin1 gene expression, combining both interventions caused a significant decrease, indicating the significant effect of using endurance training with BCAA supplementation for Parkinson's disease in rats.

Article Type: Research Article

Article History

Received: 5 July 2025
Received in revised form: 5 August 2025
Accepted: 5 September 2025
Available online:
DOI: 10.29252/jorjanibiomedj.13.X.X

Keywords

Autophagy
Autophagy-Related Protein 5
Autophagy-Related Protein 7
Beclin1
Endurance Training

Highlights

What is current knowledge?

- The prevalence of Parkinson's disease early symptoms in middle and even young ages has caused widespread global concern.
- Today, exercise training is recognized as one of the non-pharmacological methods for reducing motor, behavioral, biochemical, cardiovascular, and mitochondrial disorders in Parkinson.

What is new here?

Apparently, BCAA can be used synergistically with endurance training in Parkinson's disease to obtain better atrophy cellular effect.

Introduction

Parkinson's disease is among the most prevalent degenerative conditions affecting the central nervous system and causes slowness of movement, tremors, postural instability, loss of balance, and dysfunction of the autonomic nervous system (1). From a pathophysiological perspective, the abnormal accumulation of the protein α-synuclein in nerve cells and the formation of Lewy bodies are recognized as the hallmarks of the disease (2). Genetic and epidemiological studies have shown that there is a complex interaction between environmental factors (Such as exposure to pesticides) and genetic factors in the development of this disease (3,4). According to the World Health Organization, more than 10 million people are affected by Parkinson's disease, and as the average age of the population increases, its prevalence is expected to grow significantly in the coming decades (5). In muscle cells, the process of autophagy - a mechanism by which damaged or dysfunctional structures are broken down and repaired to maintain muscle metabolic and functional fitness - plays an essential role in maintaining cellular homeostasis and removing damaged components such as dysfunctional mitochondria and aggregated proteins (6). In general, the simultaneous activation of Autophagy related 7 (ATG7), Autophagy related 5 (ATG5), and Beclin1 is a hallmark of activation of the autophagy pathway in muscle, leading to cellular breakdown and loss of muscle mass (6). Disruption of autophagy pathways may lead to reduced muscle repair capacity, accumulation of toxic substances, and increased oxidative stress (7). Alterations in muscle autophagy activity might play a role in the deterioration of motor function, muscle weakness, and fatigue experienced by patients with Parkinson's disease (8). Therefore, studying the interaction between neurodegenerative pathways and the mechanisms regulating the autophagy process in muscle may provide new perspectives for understanding the pathophysiology of this disease and for developing targeted therapies based on regulating autophagy function.

The intersection of autophagy dysfunction may represent a critical mechanism in the pathogenesis of Parkinson's disease, in which impaired cellular clearance pathways facilitate protein transmission between cells (9). Three key proteins (ATG5, ATG7, and Beclin1) play roles in the formation of autophagic vesicles (10). ATG7 acts as an activating enzyme (E1-like enzyme) and is a prerequisite for lipid conjugation of the other two autophagy-related proteins. ATG5, with the help of Autophagy related 12 (ATG12) and Autophagy related 16 Like 1 (ATG16L1), forms an important complex that is essential for increasing the level of autophagy and the focal attachment (Recruitment) of proteins necessary for elongation of the autophagic membrane (11,12). Meanwhile, Beclin1 plays a key role in initiating the nucleation process of the autophagic membrane. The coordinated interaction of these three components is critical for the activation, initial formation, and growth of the bilayer membrane that encloses cellular material (13).

Currently, there is no definitive cure for this disease; however, the combination of medication and additional therapies, such as exercise training, can lead to significant improvements. More research is necessary to develop exercise regimens that maximize quality of life for patients and decrease mortality rates. Today, researchers have been paying increasing attention to the use of supplements alongside exercise training to control diseases, one of which is branched-chain amino acids (BCAA). The primary role of BCAAs, particularly leucine, in muscle hypertrophy (Growth) is through stimulation of the mTOR (Mammalian target of rapamycin) signaling pathway, which is the primary driver of muscle protein synthesis (MPS) (14). Consuming BCAAs, especially around the time of exercise training, can help maintain a positive net protein balance, as these essential amino acids act as fuel for muscles and reduce muscle protein breakdown (Catabolism). Although BCAA supplementation alone may not have a significant effect on muscle mass gains compared with consuming whole protein when adequate amounts of whole protein are ingested, they can be very useful as a strategy to preserve muscle mass and support recovery during caloric deficits or intense training (15).

In addition, the underlying biological and molecular mechanisms of such benefits are not fully understood; however, the antioxidant and anti-inflammatory properties of physical activity may improve the symptoms of Parkinson's disease. Recent research using mechanical devices has shown that muscle strength is diminished in patients with Parkinson's disease compared with age-matched controls, even during the early stages of the illness and on the unaffected side (15). Therefore, given the lack of comprehensive research on the simultaneous effects of exercise and BCAA supplementation, this study investigated the effect of eight weeks of moderate-intensity endurance training with BCAA supplementation on ATG5, ATG7, and Beclin1 gene expression in the soleus muscle of Parkinsonian rats.

Methods

The present experimental study was conducted in a laboratory setting with a post-test design, and the animal sample consisted of 25 male Wistar rats. The study protocol was registered with the Research Ethics Committee of Mohaghegh Ardabili University under the number IR.UMA.REC.1404.083. In this study, 25 male Wistar rats (Weight: 180-200 g, age: 12 weeks) were used as the research sample. First, the rats were divided into five groups (n = 5): healthy control (HC), Parkinson control (PC), Parkinson + continuous training (PT), Parkinson + BCAA (PB), and Parkinson + training + BCAA (PTB). The supplement groups received a dose of 300 mg/kg BCAA containing a ratio of (1-2-1 valine, leucine, and isoleucine), prepared in distilled water and administered via gavage (5 days a week) one hour before each exercise training session (16-18). All rats were housed under controlled conditions with free access to water and rat chow. The light-dark cycle was 12:12 h, and the mean ambient temperature was 22 ± 3°C.

Parkinson's induction

To induce Parkinson's disease, rotenone (Sigma, USA) was administered at a dose of 2.5 mg/kg as a daily intraperitoneal injection for 21 days. Sunflower oil was used as the solvent for the drug. Rotarod, catalepsy, and horizontal bar tests were used to confirm Parkinson's induction (19).

Method of examining the induction of the disease

To confirm the induction of Parkinson's disease in rats, the following tests were performed.

Rotation test

In this test, rats were held approximately 2 cm from the point where the tail connects to the body and raised so that the animal's nose was 2 cm above the support surface. If the rodents could not maintain their balance and turned to the sides, it was considered a sign of Parkinson's disease in the rat.

Catalepsy test

The rod test was used to evaluate muscle rigidity. This test is applied in animals in which experimental Parkinsonism has been induced to assess muscle rigidity. The device used in this test consisted of a fixed bar with a wooden platform. The height of the fixed bar from the platform was 9 cm, and the diameter of the bar was 0.9 cm. To perform the test, the rat was placed on the platform, and its two forelimbs were gently placed on the fixed bar. The time the animal remained in this position was recorded. The test was terminated when the animal removed one or both forelimbs from the bar or moved its head in an exploratory manner. Obviously, the more severe the muscle rigidity, the longer the rat remained in the imposed position (20). The rod test used to assess catalepsy is shown in Figure 1.

Figure 1. Rod test to assess catalepsy: A fixed bar with a wooden platform was used to assess muscle rigidity.

Horizontal bar

This test was performed on two 48 cm-high bases connected to each other by a 38 cm-long rod. The time required for a rat to travel 38 cm from one side to the other was recorded, and comparisons were made between different groups (21). The training step for the rotarod test involved placing rats on a rotarod with a 9 cm-diameter cylinder and a timer with an automatic touch screen for 3 minutes before the test. The number of times the rat fell off the rotating cylinder was recorded at a slow speed for 10 minutes. Rats were quickly placed back on the rotarod after each fall. The testing time for all rats was between 9:05 and 10:05 AM, and each rat was tested separately (22).

Exercise protocol

Eight weeks of endurance exercise (5 days a week) were performed on a treadmill. The moderate-intensity continuous training protocol was implemented in the first week after familiarization, with an intensity of 60 to 65 percent of maximum speed for 15 minutes, and the training duration was increased weekly. During the seventh week, the training duration increased to 30 minutes and remained the same until the end of the eighth week. In addition, the training speed remained consistent from the first week to the eighth week, set at 20 meters per minute. At the end of each session, active rest was performed at a speed of 10 meters per minute for 5 minutes. The incline of the treadmill was kept constant throughout the study (23). The procedure of the study is shown in Figure 2.

Spatial memory and learning

Spatial memory was measured using an eight-arm radial maze, which has eight identical arms that branch out radially from a small central plane that is circular in shape, with a diameter of 25 cm and a height of about 60 cm from the ground. The length of each arm is 50 cm, the width is 10 cm, and the height of the walls is 13 cm. The color of the device and cage was dark and opaque because rats are somewhat photophobic, which facilitates imaging of the rats and tracking their movements. This experiment was performed after completion of the protocol implementation period and over two days, including a training day and a test day, to observe the chronic effects of the treatment. One night before the training and test sessions, food was completely removed from the rats.

On the training day, rats were transferred to the laboratory and familiarized with the maze. Food was placed as a reward in one of the arms of the maze. During this stage, memory and learning processes were carried out without measuring the time; rats were released into the central compartment of the maze, and as soon as they found the food, they were allowed to eat a portion of it. The goal of this stage was for the rats to learn that food was located in one arm of the maze and to remember which arm contained the food.

On the second day (test day), the food was placed in the same specific arm, and the rodents were released from the center of the maze and allowed to search for the food. The time required to reach the food was measured using a stopwatch, and if a rat did not find the food within 10 minutes, it was removed from the maze. In the radial maze test, a shorter time to find the food indicates better spatial memory performance (24).

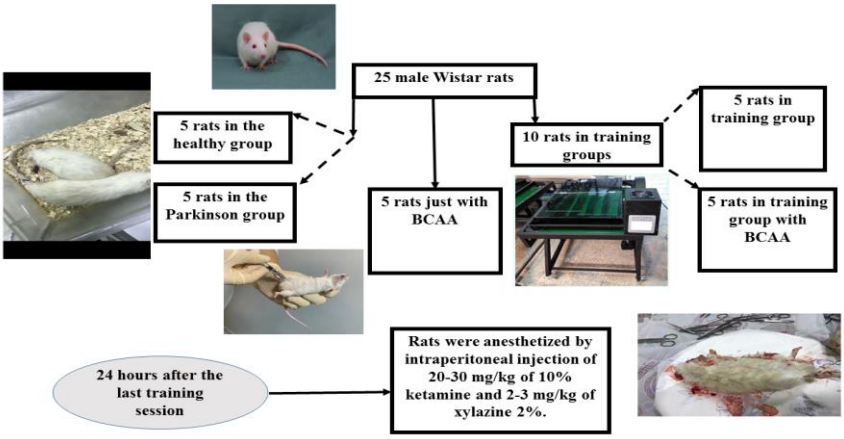

Figure 2. The grouping of rats and how they were anesthetized: 25 male Wistar rats were used as the research sample. First, rats were divided into five groups (n = 5): Healthy Control (HC), Parkinson Control (PC), Parkinson + continuous Training (PT), Parkinson + BCAA (PB), and Parkinson + Training + BCAA (PTB). Finally, 48 hours after the completion of eight weeks of exercise training and BCAA interventions, rats were anesthetized by subcutaneous injection of 20 - 30 mg/kg 10% ketamine and 2 - 3 mg/kg 2% xylazine.

The process of real-time PCR

Finally, 48 hours after the completion of exercise training and BCAA interventions, following an overnight fast, rats were anesthetized through a subcutaneous injection of 10% ketamine (20 - 30 mg/kg) and 2% xylazine (2 - 3 mg/kg). Then, the soleus tissue was isolated and, after washing with physiological saline, frozen in liquid nitrogen in RNase- and DNase-free microtubes to prevent contamination for total RNA purification and RT-PCR. The PCR product volume was 12.5 µL. Samples were centrifuged at 12,000 - 14,000 RPM at 4°C for 15 - 20 minutes. RNA extraction was conducted according to the guidelines provided by the manufacturer (Pars Tous, Iran). Thus, the samples were first stored under sterile conditions using liquid nitrogen in a porcelain mortar and kept in a -70 freezer.

The RNA extraction steps were briefly as follows: First, 750 µL of RL solution was added to microtubes containing 50 mg of powdered tissue, and the contents of the microtube were mixed thoroughly using an appropriate sampler head with repeated pipetting. Then, 200 µL of chloroform was added to each sample, followed by several rounds of pipetting. The samples were then placed in a 4°C refrigerator or on a cold rack for 10 minutes. In the next step, the samples were centrifuged at 12,000 rpm for 15 minutes. After centrifugation, three phases were formed, with RNA located in the upper colorless phase. The supernatant was carefully separated from the lower phase using a sampler and transferred to a new microtube. Then, 400 µL of cold 75% ethanol was added to the samples, and the microtubes were gently inverted and kept at 4°C for 10 minutes.

The samples were transferred to spin columns using a sampler and left at room temperature for 5 minutes. The columns were centrifuged at 12,000 rpm for 1 minute, and the supernatant was discarded. Then, 700 µL of PW wash solution was added to the columns and centrifuged at 12,000 rpm for 1 minute, after which the supernatant was discarded. Again, 500 µL of PW wash solution was added to the columns and centrifuged at 12,000 rpm for 1 minute, and the supernatant was discarded. The columns were centrifuged empty for 2 minutes at 12,000 rpm. Then, the columns were transferred to a new microtube, and 50 µL of sterile 0.05 M Tris buffer was added to elute the RNA, followed by incubation at 55°C for 5 minutes.

After that, the columns were centrifuged for 2 minutes at 12,000 rpm, and the eluted RNA sample was stored in a 1.5 ml tube at -70°C. The Easy TM cDNA synthesis kit (Pars Tos, Iran) was used to synthesize cDNA from RNA. Before preparing the cDNA reaction, the RNA samples were incubated at 50°C for 5 minutes. Then, RNA (5 µL), 2× Buffer Mix (10 µL), Enzyme Mix (2 µL), and DEPC-treated water (5 µL) were added to a 0.2 ml microtube. The components were mixed thoroughly by pipetting. The samples were then incubated at 25°C for 10 minutes, followed by 47°C for 60 minutes. To inactivate the enzyme, the samples were incubated at 85°C for 5 minutes. Afterward, the samples were stored at -20°C until the PCR reaction was performed.

Real-time PCR was used to measure mRNA expression using the Lava 96 Real-time PCR Detection System (Daan Gene Co Ltd). The kit used in the study was 2X SYBR Green Real Time PCR (Pars Tous, Iran). The Real-time PCR reaction mixture consisted of 6.25 µl of Master Mix, 0.25 µl of Forward primer, 0.25 µl of Reverse primer, 3 µl of cDNA, and 2.75 µl of water. Comparative gene expression levels relative to GAPDH expression in each tissue were evaluated using Light Cycler SW1.1 software. The results were reported based on the 2^-ΔΔCt method. Real-time PCR reactions were performed on samples as previously described, with two repetitions for each sample and for each gene, and the average Ct values of different dilutions were calculated from the two repetitions.

To validate the application of the above method, the amplification efficiency of the target gene and the internal control gene needed to be approximately equal. For this purpose, a standard curve was generated by plotting the logarithm of the standard dilution values of each gene (Log input) against the Ct values. Then, using Excel software, an XY scatter chart was created by placing the logarithm of the dilution values on the x-axis and the Ct values of each gene on the y-axis. A linear equation proportional to $y = ax + b$ was then derived, and the amplification efficiency of the target gene and the calibrator gene was calculated using the formula $E = 10 - 1/slope$. In this formula, E represents amplification efficiency. If the amplification efficiencies of the target and reference genes were similar, considering CI = 95%, the amplification efficiency of the target genes was confirmed.

For primer design, gene sequences were extracted from the NCBI gene bank, NUCLEOTIDE section, and primers were designed using PRIMER3 PLUS software. All primers were designed as exon-exon junctions, with an optimal melting temperature of 60°C and a GC content between 45 and 50% (25). The primer sequences for quantitative Real-time PCR are shown in Table 1.

Statistical analysis

Data analysis was performed using SPSS version 19 statistical software. One-way analysis of variance was used to compare changes in the indices among the four groups, and Tukey's post-hoc test was used for pairwise comparisons between groups. Statistical significance level was 0.05.

Table 1. The sequence of the primers for quantitative real-time PCR

Genes	Forward	Reverse	bp	Accession No.
ATG5	ACGGCCTTTCATTCAGAAGC	TCTTCTTCTCTCCGTCTTCAGG	122	NM_001014250
ATG7	TGGCGTTTAGCCCAGATTGT	AGCCTTTTGGGGTCCATACAT	109	NM_001012097.1
Beclin 1	AGAGTGTAGAGAACCAGATGCG	ATTGTCCGCTGTGCCAGATA	102	NM_053739.2
GAPDH	AGTTCAACGGCACAGTCAAG	TACTCAGCACCAGCATCACC	119	NM_017008.4

Results

Catalepsy, horizontal bar, and rotarod tests

The results of the catalepsy, horizontal bar, and rotarod tests are presented in Table 2. The results of the one-way analysis of variance indicated a significant difference between the groups (P-Value = 0.001). Tukey's post-hoc test revealed significant differences between the healthy control group and the Parkinson control group (P-Value = 0.001), as well as between the Parkinson + training + BCAA group and the Parkinson control group (P-Value = 0.001). The results of the catalepsy, horizontal bar, and rotarod tests are shown in Table 2.

Spatial memory and learning study

The results of the one-way analysis of variance test showed a significant difference among the groups (P-Value = 0.001). Tukey's post-hoc test indicated that, compared with the healthy control group, spatial memory and learning were decreased in the Parkinson control group. A significant difference was observed between the Parkinson control group and the Parkinson + training and Parkinson + training + supplement groups (P-Value = 0.001). Although a decrease in time (Seconds) was observed in the Parkinson + supplement group, this change was not statistically significant (P-Value = 0.152). Overall, the results demonstrated a significant improvement in spatial memory and learning in animals subjected to exercise training and exercise training with BCAA supplementation, while BCAA supplementation alone did not result in a significant improvement in memory, as shown in Figure 3.

Gene expression changes

Based on the results of the one-way analysis of variance test, a significant difference was observed among the different groups for the gene expression of ATG5 (P-Value = 0.001), ATG7 (P-Value = 0.001), and Beclin1 (P-Value = 0.001). The results of Tukey's post-hoc test showed that gene expression levels of ATG5, ATG7, and Beclin1 in the PC group significantly increased (P-Value = 0.001), with mean ± standard deviation values of 2.92 ± 0.35, 3.23 ± 0.22, and 3.31 ± 0.27, respectively, compared to the HC group 1.09 ± 0.09, 1.00 ± 0.03, and 0.99 ± 0.05, respectively, as shown in Figure 4.

The results of Tukey's post-hoc test for the PT and PB groups compared to the PC group showed a decrease that was not significant for ATG5, ATG7, and Beclin1 gene expression, respectively, with mean ± standard deviation values of 3.06 ± 0.26 and 2.27 ± 0.11, 3.23 ± 0.22 and 3.12 ± 0.23, and 3.95 ± 0.16 and 2.78 ± 0.13. A significant decrease was observed only in the PTB group compared to the PC group for the gene expression of ATG5, ATG7, and Beclin1, respectively, with mean ± standard deviation values of 1.76 ± 0.09 (P-Value = 0.001), 2.02 ± 0.07 (P-Value = 0.001), and 1.82 ± 0.21 (P-Value = 0.001), as shown in Figure 5.

Table 2. The results of catalepsy, horizontal bar, and rotarod tests

Groups / Tests	Horizontal Bars (Latency to fall (Seconds))	Catalepsy (Latency to fall (Seconds))	Rotarod Performance (Time on rod, sec)
Healthy Control	89 ± 11	73 ± 6	94 ± 6
Parkinson Control	46 ± 5*	14 ± 3*	34 ± 5*
Parkinson + Training	60 ± 7	28 ± 3	46 ± 6
Parkinson + BCAA	54 ± 6	36 ± 7	48 ± 10
Parkinson + Training + BCAA	70 ± 8**	49 ± 6**	63 ± 8**

* Indicates a significant difference between Healthy Control and Parkinson Control groups, and ** indicates a significant difference between Parkinson + Training + BCAA and Parkinson Control groups. One-way analysis of variance was used to compare changes in the indices among the four groups.

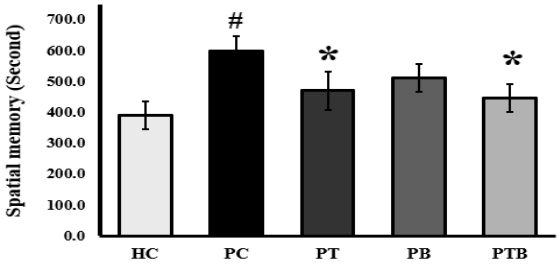

Figure 3. Differences in spatial memory and learning between the 5 study groups. The # symbol indicates a significant difference between the healthy control and Parkinson control groups, and the * symbol indicates a difference in the Parkinson + Training and Parkinson + Training + Supplement groups compared to the Parkinson control group (Abbreviations: HC, Healthy Control; PC, Parkinson Control; PT, Parkinson + Training; PB, Parkinson + Supplement; PTB, Parkinson + Training + Supplement).

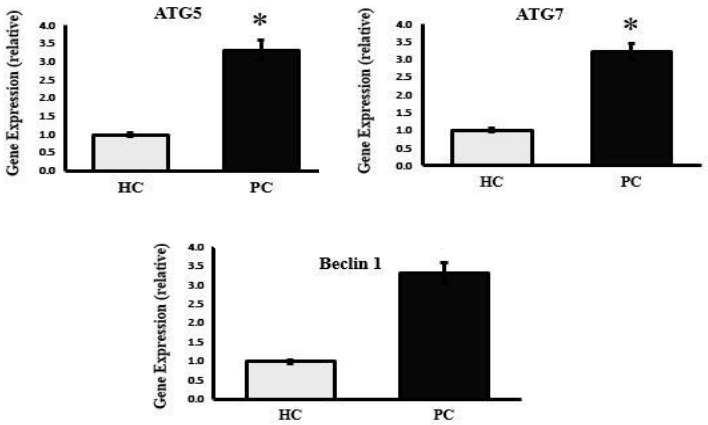

Figure 4. Mean changes in gene expression in the healthy control and Parkinson control groups. The * symbol indicates a significant increase in the expression of ATG5, ATG7, and Beclin1 genes in the Parkinson control group compared to the healthy control group (Abbreviations: HC, Healthy Control; PC, Parkinson Control).

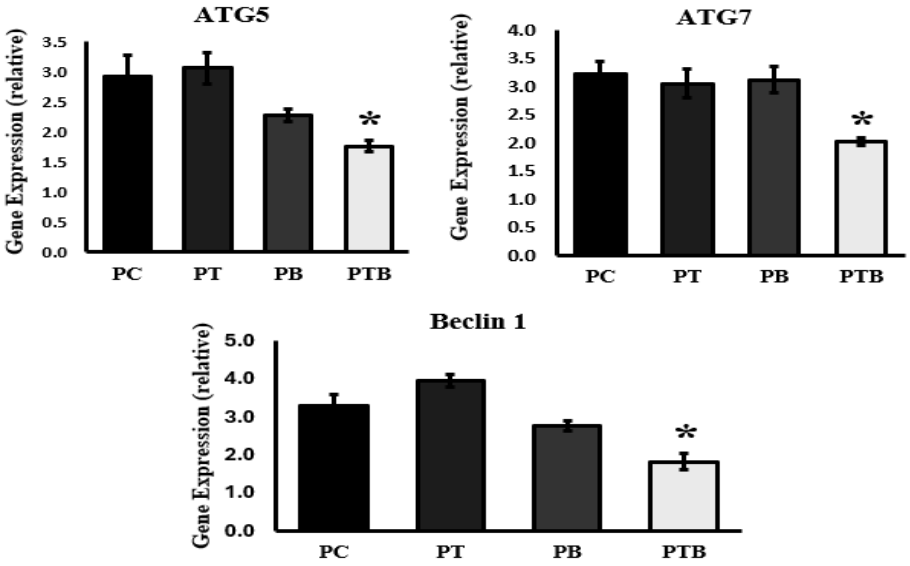

Figure 5. Mean changes in the gene expression of ATG5, ATG7, and Beclin1 in the PC, PT, PB, and PTB groups. The * symbol indicates a significant decrease in the gene expression of ATG5, ATG7, and Beclin1 in the Parkinson + Training + BCAA group compared to the Parkinson control, Parkinson + Training, and Parkinson + BCAA groups (Abbreviations: PC, Parkinson Control; PT, Parkinson + Training; PB, Parkinson + BCAA; PTB, Parkinson + Training + BCAA).

Discussion

In the present study, the effect of eight weeks of moderate-intensity endurance training with and without BCAA supplementation on ATG5, ATG7, and Beclin1 gene expression, which are associated with muscle autophagy in the soleus muscle of a Parkinsonian animal model, was investigated. The results showed that Parkinsonian induction increased ATG5, ATG7, and Beclin1 gene expression in the soleus muscle of the animal model of Parkinson's disease. Although endurance exercise and BCAA supplementation alone reduced the expression of the aforementioned genes, this reduction was not statistically significant. Eight weeks of endurance training combined with BCAA supplementation reduced the gene expression levels of ATG5, ATG7, and Beclin1. There are studies that are consistent (26,27) and inconsistent (28-30) with the results of the present study. In fact, these differences in study results may be related to variations in exercise intervention methods, the timing of sampling after exercise, the analyzed tissues, the nutritional status of the subjects prior to exercise training, the duration of exercise intervention cycles, and other factors.

In general, it has been stated that regular exercise, especially endurance exercise, is beneficial for individuals with Parkinson's disease because it reduces bradykinesia, hypokinesia, gait disorders, loss of independence, and neuronal degeneration during activities of daily living, and helps maintain cardiovascular capacity in individuals with mild to moderate Parkinson's disease (31). High-quality studies have demonstrated the benefits of aerobic exercise on balance, gait/walking, motor function, mobility, walking performance, and cardiorespiratory endurance/performance (32,33). Several studies have confirmed that exercise training, as a powerful physiological stimulus, induces the autophagy process in key tissues, especially skeletal muscle. This regulation of autophagy plays a pivotal role in the beneficial adaptations induced by exercise, such as improving oxidative capacity, maintaining muscle mass in old age, and clearing damaged organelles. ATG5, which is involved in the essential ATG12-ATG5-ATG16L1 complex, is directly associated with phagophore membrane formation and autophagosome maturation; this complex is essential for LC3-I lipidation and LC3-II generation on the membrane (34). In this regard, resistance exercise studies in aging models have shown that exercise increases the protein content of ATG5 and ATG7 in skeletal muscle, which is inconsistent with the results of the current study. This increase in gene expression, together with a decrease in LC3-II and p62 levels, suggests increased autophagic flux at basal levels as a result of chronic resistance training (34). This difference is apparently related to the type of experiment.

In Parkinson's disease, muscle cells may not receive sufficient nutrients. Although the primary pathology in Parkinson's disease involves dopaminergic neuron loss, multiple systemic factors (Inflammation, mitochondrial dysfunction, impaired autophagy, etc.) can disrupt nutrient delivery to muscle cells. This disruption leads to muscle weakness, atrophy, and overall motor dysfunction (8,35). When cells do not receive adequate nutrients, a process called bulk autophagy may be initiated. However, when specific components are marked for degradation, selective autophagy occurs. Therefore, the reduction in autophagy following exercise training, when accompanied by supplementation, demonstrates the ameliorative effect of these interventions on autophagy pathway gene expression.

Overall, it can be stated that long-term endurance and resistance training can influence autophagy-related proteins, thereby promoting skeletal muscle autophagy and slowing muscle mass loss. However, various exercise modalities - including the age at which exercise begins, as well as the duration and intensity - may have different impacts on the expression of these autophagy-related proteins. High-intensity workouts may lead to excessive autophagy, whereas short-term low-intensity exercise (Conducted for several weeks and less than three times a week) might not be sufficient to reach exercise-induced autophagy levels (36). In this regard, the study by Sadeghi et al. (2023) showed that four weeks of high-intensity interval training increased gene expression of ATG5, ATG7, and Beclin1 in the hearts of diabetic rats (30). Therefore, it is important to focus on the scientific management of chronic exercise dosage. In conclusion, studies examining the impact of chronic exercise on autophagy in aging skeletal muscle cells are still in their early stages. This indicates that the effectiveness of exercise may differ based on the type of exercise performed and the baseline levels of autophagy-related factors.

Regarding the effectiveness of BCAA, it should be noted that BCAAs have a direct effect on muscle protein metabolism. Leucine, in particular, is a potent activator of the mTOR pathway, which regulates protein synthesis. Activation of mTOR results in the translation of mRNA into proteins, particularly muscle proteins, thereby contributing to muscle hypertrophy. Conversely, when BCAA levels are insufficient, mTOR signaling may be disrupted, leading to decreased muscle protein synthesis and increased protein breakdown, processes that contribute to muscle atrophy (37). Limitations of the present study included the sample size, measurement of mRNA rather than protein levels, the use of only one sex and one muscle type, and focusing on a single dose of supplementation and exercise.

Conclusion

Exercise, especially endurance training, is a major driver of autophagy in muscle cells. The results of this study showed that, in a moderate-term training protocol, combining regular endurance training with moderate BCAA intake affected gene expression related to the autophagy process. Therefore, the overall effect of this combination depends on the type of exercise, its timing, and the dosage of the supplement.

Acknowledgement

Not applicable

Funding sources

Not applicable

Ethical statement

The study protocol was approved by the Ethics Committee of the University of Mohaghegh Ardabili (IR.UMA.REC.1404.083).

Conflicts of interest

All authors declare no conflict of interest.

Author contributions

M. Kh. played a pivotal role in statistical population selection, data collection, and laboratory coordination. M. Kh., A. R., and R. M. contributed to developing the background, and their insights and expertise were crucial to the success of this research.

Data availability statement

Not applicable

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Cite this article as:

Rajabi A, Khajehlandi M, Mohammadi R. Examination of ATG5, ATG7, and Beclin1 gene expression in the soleus muscle of animal model Parkinson following eight weeks of endurance training with branched-chain amino acids supplementation. *Jorjani Biomedicine Journal.* 2025;13(X):X. http://dx.doi.org/10.29252/jorjanibiomedj.13.X.X