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Effect of incremental resistance training on the expression of gamma acetylcholine receptor subunit genes and semaphorin-a3 in the gastrocnemius muscle of male rats

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Abstract

Background: Increasing the activity of the gamma acetylcholine receptor subunit and semaphorin a3 by affecting the transmission of nerve messages to the muscle decreases skeletal muscle function. Evidence shows that regular physical activity can promote muscle function by affecting the expression of these two genes. This study investigated the effect of increasing resistance training on the expression of acetylcholine receptor gamma (CHRNG) and semaphorin-a3 (Sema3A) subunit genes in the gastrocnemius muscle of male rats.

Methods: In an experimental study, 12 six-week-old male rats with an average weight of 195-220 grams, were randomly divided into 2 groups (6 in each group), resistance training (RT) and control (Control). The resistance training group performed incremental resistance training 5 days a week for 4 weeks. Twenty-four hours after the last training and recovery session, the sacrificial and biceps muscles of the subjects were extracted to determine the expression of CHRNG and Sema3A genes in real-time.

Results: The expression of CHRNG (P=0.044) and Semaphorine-a3 (P=0.040) genes decreased significantly in the resistance training group compared with the control group.

Conclusion: this study showed that incremental resistance training can improve neuromuscular function by reducing the expression of CHRNG and Sema3A genes at the neuromuscular junction. Based on these findings, increasing resistance exercises are recommended to improve muscle performance.

Keywords: increasing resistance training, CHRNG, Semaphorine-a3, neuro muscular junction

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Introduction

The skeletal muscle is a very heterogeneous tissue that adapts to different stimuli (1). The basis of muscle adaptability is strong neural control over force production and the regulation of the transcription and translation of genes related to nerve function (2). Force transmission from the tendon to the bone is essential for movement (1).
In addition, the skeletal muscle is a target tissue for hormonal interactions (3). Genes in the neuromuscular junction at the point where the muscle cell connects to the motor nerve terminal as transmitters of nerve messages play an important role in inhibiting cell migration, weakening strength, and reducing muscle function in causing non-morbid dystrophy (4). Inactivity can lead to the production of inhibitory proteins at the junction of the nerve and muscle, causing a decrease in the clustering of acetylcholine (AchR) (5), and due to its antiangiogenic effects, it leads to muscle atrophy. A decrease in force production and subsequent decrease in skeletal muscle function (6).
Acetylcholine receptor subunit gamma is a protein encoded by CHRNG. It affects acetylcholine production and activity (7), affects muscle contraction and muscle force production (8). On the other hand, inactivity with increasing age increases non-contracted tissue density and affects muscle strength (9). In this context, attention has been paid to the expression of a group of axonal guidance proteins called semaphorin. These proteins cause changes in the axonal function of target cells at motor nerve terminals (10). Semaphorins are expressed in the nervous, immune, and cardiovascular systems (11).

Among the semaphorin group, Semaphorin-a3 is expressed in the neuromuscular terminals of type 2 (II) fibers, causing axon dysfunction due to the effect of neuropilin-1 (NRP-1) and plexin-a (PLX-A) receptors. (12), it inhibits nerve conduction towards the muscle cell and reduces muscle strength by decreasing force production. Studies have shown that force production inhibitors affecting neuromuscular connections are more effective in fast-twitch fibers due to the higher presence of acetylcholine terminals (13). An increase in these inhibitory factors affects force production and strength. Regular physical activity is a non-invasive protective mechanism against various diseases and helps maintain the structure and function of the synapse, preventing diseases related to the neuromuscular system. The effect of exercise varies based on its basic characteristics (intensity, duration and type) and the volume of contracting muscles (14,15), leading to different and sometimes contradictory results. Resistance training, such as weight lifting, can enhance pre- and post-synapse components in the neuro-muscular connection. This type of exercise increases tension in the pre-synaptic area due to the use of increasing loads and tissue metabolites in fast fibers, leading to expansion in the post-synaptic area during recovery (16,17,18). Endurance training at the neuromuscular junction (NMJ) enhances the speed of nerve message transmission to skeletal muscle by promoting mitochondrial biogenesis and increasing the production of calcitonin gene-related factors in motor neurons' cell bodies (19,20).

Endurance training improves the neuromuscular junction and the cross-sectional area of the nerve-muscle connection by activating acetylcholine receptors and producing acetylcholinesterase (21). According to past review studies, acetylcholinesterase activity values are higher in fast-twitch fibers than slow-twitch fibers (7). Resistance training increases the specific tension of the contracting muscle by enhancing the availability of sodium and calcium ions. It also releases acetylcholine from the terminal. It rapidly increases nerve impulses and facilitates more neuromuscular connections (22).Also, in resistance training, resting between intense repetitions is a more important factor that causes the hypertrophy

pathway to be launched through the stimulation of the motor nerve in fast-twitch muscles and strength improvement (23) and compared to endurance training with lower intensity and duration. For a longer period, resistance training is effective by increasing the call of type II filaments in the activation of myosin heavy chain (MHC) isoforms and increasing the quantitative content of vesicles and reducing synaptic fatigue (24). Evidence shows that exercise intensity is a more effective factor in improving gene expression, preventing mutations, modulating gene transcription and translation improves enzyme activity and protein synthesis. This affects the transmission of messages from the motor nerve to the muscle (17). Regeneration of damage in the area of nerve-muscle connection due to tolerance and repetition of mechanical overload in blood flow limitation, temporary hypoxia, endocrine responses and accumulation of metabolic substances such as lactic acid and adenosine diphosphate (ADP) have resulted (25) And in the recovery period after training, it has a great effect on the production of neutrotrophins and neuromuscular efficiency (26). According to studies conducted in the first weeks of strength training, more neural adaptations appear, which precede muscle hypertrophy (22). However, most of the studies conducted on resistance training have focused on clinical models (4) and the study on the effect of intense resistance training on the improvement of neuromuscular function in healthy subjects is small, so the present study The effect of 4 weeks of increasing resistance training on the expression of CHRNG and Semaphorine-a3 genes in the gastrocnemius muscle of healthy male rats has been studied.

Materials And Methods

Subjects

In an experimental trial, 14 male Wistar rats, weighing between 195 and 220 grams, were purchased and transferred to the animal laboratory. Table 1. After a week of familiarization with the laboratory environment, the subjects were randomly divided into two groups of 6 including increasing resistance training (RT) and control (C). The rats were kept in transparent polycarbonate cages manufactured by Razi Rad Company. They were kept in an environment with a temperature of 22±2 degrees Celsius and a light-dark cycle of 12:12 with free access to water and animal food (pellets). All stages of the study were carried out in accordance with laboratory animal principles.

Incremental resistance training program

A week after the subjects have been familiarized with the resistance training program (ladder of 110 cm height, with 2 cm spacing between the rungs and an 80 percent slope) was used to implement the increasing resistance training program. With the trainer's help, they went up the stairs 3 to 5 times with high repetitions without carrying weights. A maximum repetition (1RM) was performed by adding weights with Lecoplast adhesive to the tails of the rat before the increase in resistance training protocol was implemented (the rat's tails were tested for sensitivity to this type of adhesive before the exercise began).

In the first session, the training started by adding a weight equal to 50% of the body weight to their tail, then 30 grams of weight was added to the Hurst and continued until the subjects could

not lift the weight. According to this, the last weight the subjects carried was considered to be the maximum number of repetitions that they could perform (27). The resistance training program was for 4 weeks and 5 days a week. The sixth day of each week was considered to be the maximum number of repetitions to be performed for a gradual increase in weight for the following week. Before the exercise, they first performed the warm-up program in 3 repetitions without carrying weights, then resistance exercise was done in the first week with 50% of the subjects' body weight, and for the next sessions, the exercise was started with 50% of the last weight lifted. Accordingly, the training load was 50% in the first week, 75% in the second week, 90% in the third week, and 100% in the fourth week. This was the maximum weight they carried on the ladder.The number of repetitions in each session was 2 repetitions and in 3 sets with a rest time of 1 minute between each repetition and 2 minutes between each set (27). During this period, to equalize, the control group was placed on the ladder 5 times a week for 10 to 15 minutes each session.

Animal sacrifice, tissue removal

In the fourth week, 24 hours after the last training session and recovery after that, the rats were anesthetized by intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). Then, a blood sample was collected directly from the left ventricle of the heart of the rat to cause death. Then, the gastrocnemius muscle tissue was immediately extracted by cutting the lower limb. It was frozen in -20 nitrogen and stored in a -80 freezer for gene expression measurement.

Measuring gene expression

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To measure the expression of CHR-G and Sema3A genes, Realtime-PCR method was used with Premix Extaqit and GAPDH was used as the control gene, and the expression value of this gene was measured in combination with each of the genes with the 50 Mirnasy mini kit. The kit (made by Qiagene in Germany) was prepared according to the recipe. For RNA extraction, 50 mg of frozen rat gastrocnemius muscle tissue was homogenized. According to the manufacturer's instructions, RNA solution was extracted from it and purified from DNA contamination and RNA degrading enzymes by DNaseI enzyme. From each sample, 2 micrograms of mRNA were used to synthesize the first strand of cDNA. The relative amount of gene expression for the studied genes in the gastrocnemius muscle was measured with their specific primers. The absorbance ratio of 260 to 280 nanograms was 1 to 2.8 for all extracted samples. To check the quality of extracted RNA, electrophoresis and 1% agarose gel were used. It should be noted that DNAs treatment (Thermos Scientific, made in Germany) was done to ensure the absence of DNA in the extracted sample before a cDNA assay. cDNA synthesis was carried out using the first strand cDNA synthesis kit (Roch, Germany) according to the instructions of the kits.

Real time PCR analysis was performed with Rotrogene 6000, Corbet, Germany. According to Syber Green (Ampligon, Denmark), this program consisted of a cycle of 95°C for 15 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds with designed primers (manufactured by Nika Biogen, Iran). It is presented in Table 1.

Table 1- Primers used.

Gene name	Forward	Reverse
CHRNG	AGAGAATGGTCCAGAAATGAG	GCTAGGAAACAGACACGGT
Sema3A	CTACTGGACATTTCTTTGGTC	GGCTCCTGCTTCGTAGTCT
GAPDH	AAGTTCAACGGCACAGTCAAGG	CATACTCAGCACCAGCATCACC

CHRNG: Acetylcholine receptor subunit gamma, Sema3A: Semaphorine-a3, GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase.

Statistical model

Data obtained from genetic assays are reported based on the mean and standard deviation. Shapiro-Wilk test determined normal data distribution. The difference between increasing resistance training and control groups was analyzed using a t-test for independent groups. A significance level of ≥5% was considered. All calculations were performed using Graph Pad Prism version 8.

Results

After 4 weeks of resistance training, the subjects' weights did not change significantly (Table 2).

Pre- and post-test weights of subjects in the progressive resistance training group and the control group. Standard deviation and mean are reported for data.

P value	progressive resistance training	Control		
P=0.124	209.19±10.37	205.16±14.41	Pre-test	(grams) weight
P=0.698	208.21±12.73	207.46±12.62	Post-test	weight (grams)

Compared to the control group, progressive resistance training decreased the expression of the CHRNG gene in the gastrocnemius muscle (P=0.044) Figure 1.

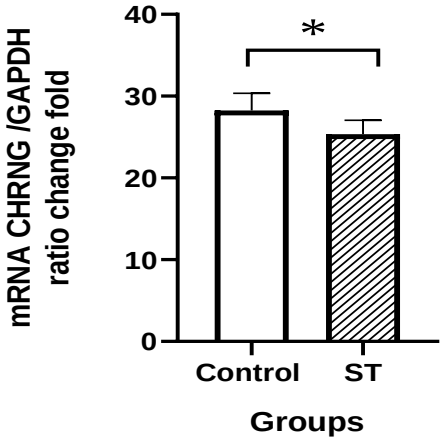

Figure 1: The ratio of CHRNG gene expression to GAPDH in the progressive resistance training group and the control group.

*Significant difference compared to the control group (P=0.05). Information is reported based on the mean and standard deviation.

Progressive resistance training significantly decreased the expression of the Sema3A gene in the gastrocnemius muscle compared to the control group (P=0.040) Figure 2.

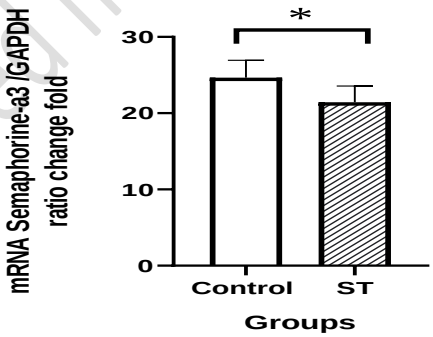

Figure 2: Sema3A gene expression ratio to GAPDH in the progressive resistance training group and the control group.

*Significant difference compared to the control group (P=0.05). Data are reported based on mean and standard deviation.

Discussion

The present study investigated the effect of 4 weeks of resistance training on the expression of CHRNG and Sema3A genes in the gastrocnemius muscle of healthy male rats. According to the findings, the expression of CHRNG and Sema3A genes in the progressive resistance training group decreased significantly compared to the control group. However, there was no significant difference in weight. In order to improve neuromuscular function, exercise intensity and duration are key factors (17).

On the other hand, using a large muscle mass in training creates a greater metabolic cost than training with a smaller muscle volume. It also brings a higher metabolic response in the microcellular and molecular parts and higher functional responses (5). Therefore, high-intensity exercise by using large muscles in the call and increasing the activity of fast-twitch fibers causes an increase in the response of K+ and Na+ ions along with calcium, causing more release of acetylcholine from the nerve terminals to the ends of the muscle fibers. And the response creates more neuro-muscular adaptation (22). Since the speed of transmission of stimulation messages from the axon to the end of the muscle fiber involved in the activity has increased, more force is produced by the muscle (20). For this reason, during repeated contractions with increased mechanical load tolerance, temporary limitation in blood flow and subsequent temporary hypoxemia, the temperature of the muscle involved in the contraction increases and due to the increase in metabolites caused by contraction such as lactic acid and adenosine, the production and release of acetylcholine expands the presynaptic space (18). But during recovery time after training, by producing dilators such as nitric oxide and prostaglandins, it causes more blood supply to the muscle. By activating the neutrophins at the end of the thread, the production of acetylcholinesterase expands the postsynaptic space. He gives (19). Also, the implementation of a strength program using type II muscles creates special muscle tension, and on this basis, compared to submaximal training performed for a longer period of time, it brings less fatigue and these exercises can be used for people with health levels and with different physical fitness (24). Also, fast-twitch fibers in skeletal muscles have androgenic receptors, which are used in strength training, and after training, they cause growth factors to be called and prevent muscle destruction (28). It has been reported that 12 weeks of aerobic exercise on a treadmill has no effect on the distribution of α-acetylcholine receptors in the neuromuscular junction (29). While it has been reported that combined training (strength-endurance) has caused a higher increase in the number of acetylcholine receptors in fast and slow twitch fibers than the other two types of training because it increases the function of calcitonin receptors in CGPR at the end of the driving plate. The muscle fiber contracts and increases acetylcholine receptor function (30). In examining the response of different exercise patterns to the changes of atrophy factors in the gastrocnemius muscle of Spirogudauli mice, it was reported that endurance exercise on a treadmill and limb suspension group both resulted in a significant decrease in the weight of the twin muscle along with an increase in the expression of muscle tissue-destroying genes, including Murf and Foxo showed, while no difference in muscle weight and expression of genes involved in the atrophy process was observed in the external resistance training group (31).

Sukho et al. results's (2003) on the effect of 8 weeks of strength training on a ladder with 26 steps with a vertical angle of 85 degrees, 5 days a week. The ladder started with 30% of body weight in the first week and reached 200% in the last week. , did not observe a difference in

the mass of the soleus and quadriceps muscles, but the mass of the flexor muscles of the toes increased (23). The results of Al-Gadi et al. (2015) on the effect of 8 weeks of progressive resistance training for 3 days a week by carrying weights of 50, 75, 90 and 100 percent of body weight, the amount of agrin protein and the amount of acetylcholine in the soleus muscle compared to the group Control was significantly increased and it was reported that resistance training with gradual overload creates higher adaptations in the call of muscle nerve in improving force production and muscle strength (32). It has been reported that 4 weeks of HIIT training and 5 sessions per week decreased the expression of the Sema3A gene in the long toe muscles of old mice. One of the reasons is the use of fast-twitch strings in intense cycles (33). According to the findings of the present study, increasing resistance training did not make a difference in the weight of the subjects. One of the reasons could be the length of the training period. However, four weeks of increasing resistance training caused a significant decrease in the expression of CHRNG and Sema3A genes in the nerve junction of the gastrocnemius muscle of rats. Based on these changes, the pre-synaptic space is expanded and improves neuromuscular function. We can describe the mechanism of training intensity influencing the expression of the mentioned genes in improving neuromuscular function (22) according to the theory of size and motor nerve control. Based on the principle of size in calling the motor units, the nervous system activates the small motor units in the muscle to perform weaker and smaller contractions. By increasing the load, it stimulates the larger motor units, so this is one of the solutions. With resistance exercises, large motor units that may have been less activated before are activated more and muscle strength increases.

Controlling the motor nerve by determining which and how much motor units need to be activated to produce maximum force. As a result, resistance training can produce more muscle force through the excitation and activity of larger motor units (34). This shows that resistance training is an increasingly effective factor in regulating the expression of genes involved in nerve message transmission (17). However, more studies are still needed in this field. In these studies, the influencing factors on neuromuscular junctions were investigated at the gene level. One of the limitations of this study is the lack of measurement of histological characteristics of nerves and muscle. This is suggested to be studied to clarify the exact effect of resistance training on neuromuscular junction factors.

Conclusion

The results of this study showed that four weeks of increasing resistance training decreased the expression of CHRNG and Sema3A genes in the neuromuscular junction of the gastrocnemius muscle. This, considering the role of these genes, can improve neuromuscular function. Based on these findings, increasing resistance exercises affect skeletal muscle function by influencing the effector genes at the neuromuscular junction. However, more studies are needed in this field.

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Conflict of interest

There is not conflict of interest.

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