Journal of Sports Physiology and Athletic Conditioning (J.S.P.A.C) 2024; 4 (11): 1-9

Research Article

Effect of 4 weeks of resistance training on Neural cell adhesion molecule gene expression of neuromuscular junction, gastrocnemius muscle in male rats

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Received: 14 December 2023
Revised: 20 January 2024
Accepted: 1 March 2024

Abstract

Background: Resistance training improves skeletal muscle function by affecting the proteins of the nervous system. However, there are conflicting results regarding the effects of resistance training on Neural cell adhesion molecule (NCAM) gene expression. Therefore, the present study aimed to investigate the effect of 4 weeks of increasing resistance training on NCAM gene expression in the gastrocnemius muscle of healthy male rats.

Materials and Methods: In an experimental trial, 12 young male rats were randomly divided into 2 groups of 6, including the control and resistance training groups. The training group performed increasing resistance training 5 days a week for 4 weeks on a special rodent ladder. Forty-eight hours after the end of the training intervention, the rats were sacrificed and the gastrocnemius muscle tissue was extracted for the expression of the NCAM gene using the real-time method.

Results: resistance training in the neuromuscular junction, gastrocnemius muscle increased NCAM gene expression (P=0.036) compared to the control group.

Conclusion: Four weeks of resistance training can improve skeletal muscle function by increasing NCAM gene expression at the end of muscle fibers.

Keywords:
Resistance training, Neural cell adhesion molecule gene

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1. Introduction

Skeletal muscle is very important due to the presence of fast and slow contraction fibers in the process of force generation and regulation of transcription in the translation of expression of genes related to motor nerve function (1). At the end of the skeletal muscles, important genes cause the transmission of nerve messages to the depth of the muscle tissue (2) and control the function of the muscle due to Bertolide's effect and force transmission to the tendon (3). Muscle response at different levels of force production is directly related to the principle of muscle fiber recruitment and the volume of muscle use (4). Improper changes in the transcription process and mutation of genes related to the decrease in muscle activity cause a decrease in strength and subsequent weakening of strength (5). Inactivity and lack of regular physical activity increase the density of non-contractile tissues (6). Often, due to the decrease in acetylcholine production and disturbance in calcium release, the process of exocytosis is disturbed and together with the synthesis of inhibitory proteins at the end of the motor nerve, force production also decreases (7). However, during the muscle activity along with the contracting process, the expression of a special type of nerve adhesion molecule (NCAM), which is from the family of immunoglobulins, inhibits the atrophy process (11). Also, the expression of this type of protein improves axonal function and develops the hypertrophy process (12). The first step in creating hypertrophy in skeletal muscle cells is the growth and repair of neurons, which is related to the expression of growth factors and nerve conduction (13). According to the conducted studies, resistance training causes the activation of nerve units at the end of the stimulator plate of the muscle tissue and improves strength (14).

Also, resistance training can increase the production and release of Ca++ from the cytosol of the cell, and due to the activation of protein kinase alpha, beta, and gamma isoforms, as well as calcium-dependent kinase such as calmodulin, it can directly increase protein synthesis in skeletal muscle. develops (15). Therefore, due to the use of fast-twitch fibers along with strength training, it seems that doing this type of training is effective for maintaining the neuromuscular structure and function in preventing related diseases (16). A comparison of the effect of the type of exercise in preventing atrophy showed that resistance exercise is more effective than endurance exercise on pre- and post-synaptic components in expanding nerve-to-muscle connection (17). Resistance training increases muscle tension due to the faster release of Na+, and Ca++ ions, the release of acetylcholine from nerve terminals, and creates neuromuscular relaxation (18). On the other hand, during resistance training, contracting muscles are subjected to mechanical overload, which stimulates the production of myosin heavy chain, and increases the number of vesicles, improves the speed of nerve message transmission at the end of the muscle fiber (19). Also, the increased cell metabolism during resistance training with increasing intensity, through the activation of the insulin signaling pathway and insulin-like factor-1, smooths the process of protein synthesis, followed by hypertrophy (15). Also, resting between sets of resistance training after motor nerve stimulation is another stimulus in the path of hypertrophy in axon sprouting and its transfer from the extracellular matrix (20). For this reason, resistance training leads to the development of skeletal muscle hypertrophy by calling motor nerve units directly (21) and indirectly by using cellular metabolism (15).

It has been reported that resistance training with appropriate volume increased neural activity (4). Based on this, considering the beneficial effect of resistance training on improving muscle strength, and the existence of contradictions in the findings of previous studies, the present study aimed to investigate the effect of 4 weeks of increasing resistance training on NCAM gene expression in the neuromuscular junction of the gastrocnemius muscle of healthy male rats.

2. Materials and Methods

In an experimental trial, 12 male Wistar rats weighing between 185 and 220 were purchased from the Institute Pasteur in Iran and transferred to the animal laboratory. After a week of familiarization with the laboratory environment, the subjects were randomly divided into two resistance training groups and the control group. The subjects were kept in transparent polycarbonate cages made by the Razi Rad Company in an environment with a temperature of 22±2 degrees Celsius and a light-dark cycle of 12:12 and free access to water and food for animals (pellets). All stages of the study were carried out in compliance with the principles of working with laboratory animals approved by the Ministry of Health and Medical Education of the Islamic Republic of Iran.

Resistance training program

To implement the increasing resistance training program, after a week of familiarizing the subjects with the resistance training program (ladder with a height of 110 cm, the distance between each step is 2 cm and the slope of the ladder is 80 percent) without carrying weights with the help of the trainer, 3 to 5 high repetitions They went up the stairs.

Before the implementation of the increasing resistance training protocol, the maximum of one repetition (1RM) of the subjects was performed by adding weights to the tail with Lecoplast adhesive (the sensitivity of the mice's tail to this type of adhesive was checked before the exercise). 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 sets and continued until the subjects were unable to lift the weight, according to this, the last weight that the subjects carried was considered as a maximum of one maximum repetition (22). The resistance training program was carried out for 4 weeks and 5 days a week, and the sixth day of each week was considered to measure the maximum of one repetition of gradual weight gain for the following week. Before the exercise, they first performed the warm-up program in 3 repetitions without carrying weights, then resistance exercise was performed 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 carried. In this way, the training load included 50% in the first week, 75% in the second week, 90% in the third week, and 100% in the fourth week of the maximum weight that they managed to carry 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 (22). During this period, for equalization, the control group was placed on the ladder 5 times a week for 10 to 15 minutes in each session. The details of the resistance training program are presented in Table 1.

Table 1. training protocol

Resistance training				
4	**3**	**2**	**1**	**Week**
100%	90%	75%	50%	Amount of weight per session (1RM)
3	3	3	3	The number of sets per session
2	2	2	2	Number of repetitions per set per session
2	2	2	2	Rest time between sets(min)
1	1	1	1	Rest time between each repetition (min)

Tissue sampling And RNA extraction and quantitative real-time PCR

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 the blood sample was collected directly from the left ventricle of the heart of the mice to cause the death of the subjects. Then, the tissue of the gastrocnemius muscle was immediately extracted by cutting the lower limb frozen in -20 nitrogen and stored in a -80 freezer for gene expression measurement.

To measure NCAM gene expression, the real-time method with Premix Extaqit was used and GAPDH was used as a control gene, and the expression value of this gene was measured in tandem with each of the genes with a 50 Mir nasy mini kit (manufactured by Qiagen). Germany), performed according to the instructions. For RNA extraction, 50 mg of frozen rat gastrocnemius muscle tissue was homogenized and according to the instructions of the manufacturer of the kit, the RNA solution was extracted from it and cleaned from any DNA contamination and RNA degrading enzymes by DNaseI enzyme.

For each of the samples, 2 micrograms of mRNA were used to synthesize the first strand of cDNA. The relative amount of gene expression of the studied genes in the twin muscle was measured with the help of their specific primers, and the absorbance ratio of 260 to 280 nanograms was 1 to 2.8 for all extracted samples. Checking the quality of RNA extracted by electrophoresis and 1% agarose gel was 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 cDNA assay. cDNA synthesis was done using transe criptor first strand cDNAsynthesis kit (Roch, Germany) according to the instructions of the kits. A real-time PCR program was performed with a Rotrogene 6000, Corbet, made in Germany. The program according to Syber Green (ampligon, made in Denmark) with a cycle of 95°C for 15 minutes and immediately 40 cycles with 95°C for 15 seconds and 60°C for 60 seconds with design primer (Made by Nika Biogene Iran) was done. Gene expression quantification was calculated with the formula ΔΔ ct-2 and Fold Change values. The primers used are presented in Table 2.

Table 2. Primers used

Gene name	Forward	Reverse
NCAM	CTACTGGACATTTCCTTGGTC	GGCTCCTGCTTCGTAGTCC
GAPDH	AAGTTCAACGGCACAGTCAAGG	CATACTCAGCACCAGCATCACC

NCAM: Neural cell adhesion molecule: GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase.

Statistical model

Data obtained from genetic assays are reported based on mean and standard deviation. The normality of data distribution was determined by the Shapiro-Wilk test. Determining the difference between 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 the software GraphPad prism version 8.

3. Results

NCAM gene expression in neuromuscular junction, gastrocnemius muscle increased significantly after resistance training compared to the control group (P=0.036) Figure 1

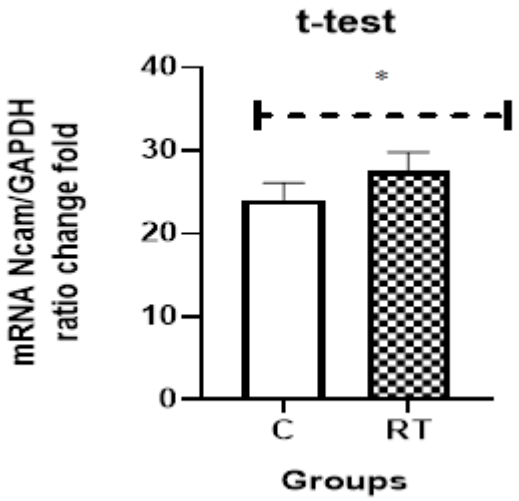

Figure 1: The ratio of NCAM gene expression to GAPDH in the increasing resistance training group and the control group. *Significant difference compared to the control group (P=0.05). Data are reported based on mean and standard deviation.

4. Discussion

In the present study, the effect of 4 weeks of increasing resistance training on NCAM gene expression in the gastrocnemius muscle of male rats was investigated. The results showed that NCAM gene expression increased significantly in the training group. Resistance training by influencing the process of sodium and potassium channels in the muscle fiber increases the speed of nerve message transmission and by releasing more acetylcholine from motor nerve terminals, it creates a greater neuro-muscular response and creates a stronger contraction in the muscle. represent (23,24). On the other hand, during repeated contractions, with mechanical overload, blood flow restriction subsequent lactic hypoxemia, and increased adenosine, the production and release of acetylcholine in the presynaptic environment expand (25). The fast-twitch fibers in skeletal muscles have androgenic receptors, which in response to strength training, prevent the destruction of the muscle structure by activating protein synthesis signaling pathways (26). During the recovery after exercise, the increase in nitric oxide causes more blood supply to the muscle and activating neurotrophins regulate the synthesis of acetylcholinesterase and develop the function of the neuromuscular junction (27). It has been reported that combined training (strength-endurance) causes an increase in the number of acetylcholine receptors in fast and slow twitch fibers compared to strength and resistance training, as well as the content of acetylcholine in the motor nerve, which increases the contraction performance (28,29). NCAM is a member of the family of immunoglobulins and is expressed in nerve cells and glial cells, which is closely related to axonal growth, remyelination processes, guidance, and fasciculation, and participates in repair mechanisms (A).

The improvement in muscle strength induced by resistance training may be partly explained by the reinnervation of skeletal muscle fibers and upregulation of NCAM (B). An increase in MHC II fiber size after resistance training has been reported to be associated with increased NCAM gene expression (C). MHC II fibers have been reported to be at risk of inactivity-related atrophy, and resistance training preferentially increases MHC II fiber function and size. Increased NCAM expression in MHC II fibers is more prominently associated with resistance training-induced improvements in fiber strength and size. Based on this, it is clear that improvement in fiber diameter and total muscle strength after resistance training may be achieved through neural adaptations/muscle fiber reinnervation. Evidence shows that the decrease in NCAM expression in experimental autoimmune encephalomyelitis mice may be attributed to increased inflammation and oxidative stress in the neuromuscular junction as well as axonal damage. On the contrary, in mice with experimental autoimmune encephalomyelitis, NCAM expression was increased by exercise, which seems to be due to the reduction of inflammation and oxidative stress caused by exercise (D). Based on this, it seems that the resistance exercise used in the present study may have increased the expression of NCAM in the neuromuscular junction by inhibiting inflammation and oxidative stress. NCAM plays an important role in signal transmission, synaptogenesis, synaptic plasticity, promotes and regulates synaptic stability, and strongly affects neurotransmission (E). According to the roles of NCAM, its increase after resistance training is one of the mechanisms for improving the performance of the squat muscle due to neurological changes. It has been reported that intense intermittent exercise prevented muscle atrophy by increasing

NCAM in old rats (12). According to the results of the present study regarding the effect of resistance training on the increase of NCAM in the gastrocnemius muscle of healthy mice, this type of training is probably effective in expanding the presynaptic space.

Conclusion

The results of the present study showed that resistance training increased NCAM gene expression in the gastrocnemius muscle. Since NCAM gene expression is associated with increased synaptic stability and improved nerve transmission, it can be concluded that resistance training by influencing the expression of this gene improves the process of transmitting nerve messages to skeletal muscle and thereby improves muscle function.

Acknowledgements

The researchers hereby express their gratitude and thanks to the research subjects.

Funding

This study did not have any funds.

Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: M.H, M.A.A, Sh.R.M, M.P, M.H.H; Methodology: M.A.A, Sh.R.M, M.P, M.H.H; Software: M.H, M.A.A, Sh.R.M, M.P; Validation: M.H, Sh.R.M, M.P, M.H.H; Formal analysis: M.H, M.A.A, M.P, M.H.H; Investigation Sh.R.M, M.P, M.H.H; Resources: M.H, M.A.A, M.H.H; Data curation: M.H, M.A.A, Sh.R.M,; Writing - original draft: M.H, M.P, M.H.H; Writing - review & editing: M.H, M.A.A, Sh.R.M, M.P; Visualization: M.A.A, Sh.R.M, M.P, M.H.H; Supervision: M.H, M.A.A, M.H.H; Project administration: M.H, M.A.A, Sh.R.M, M.P, M.H.H; Funding acquisition: M.H, M.A.A, Sh.R.M, M.P, M.H.H.

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