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Original Article

Long-term Low-Intensity Endurance Exercise along with Blood-Flow Restriction Improves Muscle Mass and Neuromuscular Junction Compartments in Old Rats

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What's Known

- Aging-induced neuromuscular junction (NMJ) remodeling reduces nerve terminals and nicotinic acetylcholine receptors, inevitably leading to motor imbalance.
- A completely safe and effective therapeutic method to prevent NMJ downregulation has yet to emerge.

What's New

- We examined the effects of low-intensity aerobic training along with limb blood-flow restriction as a safe procedure on acetylcholine receptors clustering at the NMJ in old rats.
- Results showed the beneficial effects of this method in protecting and improving muscle mass and the NMJ.

Abstract

Background: During the aging process, muscle atrophy and neuromuscular junction remodeling are inevitable. The present study aimed to clarify whether low-intensity aerobic exercise along with limb blood-flow restriction (BFR) could improve aging-induced muscle atrophy and nicotinic acetylcholine receptors (nAChRs) at the neuromuscular junction.

Methods: Forty-eight male Wistar rats, aged 23–24 months, were randomly divided into control, sham (Sh: subjected to surgery without BFR), BFR (subjected to BFR), exercise (Ex: subjected to 10 weeks of low-intensity exercise), Sh+Ex, and BFR+Ex groups. Forty-eight hours after the last training session, the animals were sacrificed and their soleus and extensor digitorum longus (EDL) muscles were removed. The hypertrophy index was calculated, and molecular parameters were measured using western blotting. Statistical analysis was done with ANOVA using SPSS (version 20), with a $P<0.05$ as the level of significance.

Results: The control and Sh groups showed weight gain ($P=0.001$), whereas the Ex, Sh+Ex, and BFR+Ex groups had significant weight loss ($P<0.001$). The hypertrophy index of the soleus was significantly higher in the BFR+Ex group than in the control, Sh, and BFR groups ($P<0.001$). BFR+Ex induced significant hypertrophic effects on the EDL ($P<0.001$ vs. the control, Sh, Ex, and Sh+Ex groups, and $P=0.006$ vs. the BFR group). BFR+Ex also increased nAChRs in the soleus ($P=0.02$ vs. the control and Sh groups) and the EDL ($P=0.008$ vs. the control and Sh groups).

Conclusion: BFR plus mild exercise is a safe method with potential beneficial effects in protecting and augmenting muscle mass and nAChR clustering at the neuromuscular junction in old rats.

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Keywords: ● Aging ● Blood-flow restriction ● Exercise ● Nicotinic acetylcholine receptors ● Neuromuscular junction

Introduction

The aging process is concomitant with such alterations as reductions in the number of myelinated nerve fibers, nerve-fiber

diameter, and motor nerve conduction velocity[1] as well as remodeling in the neuromuscular structure and loss of its performance.[1] Such deterioration manifests itself as a defect in physical function and loss of independence.[1] Aging-induced neuromuscular junction (NMJ) remodeling entails reductions in the number of presynaptic vesicles, nerve terminals, and nicotinic acetylcholine receptors (nAChRs) together with an increase in the space at the motor end plate.[2] The remodeling of motor units is preceded by the denervation of motor units, especially type II fibers. The final pattern is the conversion of type II fibers to type I fibers and an increase in type I fibers.[2] Further, morphological changes at the NMJ in older people are associated with the greater fragmentations of nAChRs and nAChR clustering.[3]

Physical activity is effective in preventing and slowing down the aging process while maintaining muscular strength and endurance.[4] It is reported that both resistance training and endurance training are allied to a significant increase in the number of the presynaptic and postsynaptic components of the NMJ.[5] Aerobic training is known as an intervention that improves muscle function, NMJ, metabolism, and motor-unit adaptability.[6] Moreover, physical activity plays a role in delaying age-related loss of muscle mass.[6]

Recently, the KAATSU exercise method has been developed as a combination of exercise training and blood-flow restriction (BFR).[7] When a low-intensity resistance exercise (e.g., 20% of 1-repetition maximum) is combined with BFR, it significantly augments muscle size, strength, and endurance beyond what could possibly be gained by contractive exercise.[7,8] On the other hand, aerobic training (e.g., treadmills or bicycles) is known to improve cardiopulmonary endurance, which is an important aim of physical therapy.[9] Previous studies have confirmed the positive effects of endurance training on nAChRs at the NMJ of adult rats.[6,10]

Old persons often develop motor weakness and strength loss and cannot do heavy exercise to maintain strength and endurance.[11] What could also prove problematic for the elderly is the fact that high-intensity resistance training increases blood pressure.[9] Given the higher safety of low-intensity endurance exercise in old age, we hypothesized that low-intensity aerobic training combined with BFR might recover the clustering of nAChRs and improve the performance of the NMJ. Because of the paucity of relevant research in the existing literature, in the present study, we used old rats to examine the effects of 10-weeks of low-intensity aerobic training combined with BFR on hypertrophy response and nAChRs in 2 types of muscles: fast-twitch (the extensor digitorum longus [EDL]) and slow-twitch (the soleus).

Materials and Methods

The present study was conducted in 2016 in Kerman University of Medical Sciences, Kerman, Iran.

Animals

Forty-eight male Wistar rats, aged 23–24 months and weighing 400–450 g, were randomly divided into 6 groups (8 in each group): the control (CTL) group, which received care without any surgery and exercise training; the sham (Sh) group, which was subjected to surgery without BFR; the BFR group, which was subjected to surgery and restriction of the hind blood flow during activity; the exercise (Ex) group, which did prolonged low-intensity treadmill exercise; the Sh+Ex group, which underwent surgery without BFR and did prolonged low-intensity treadmill exercise; and the BFR+Ex group, which underwent surgery with BFR and did prolonged low-intensity treadmill exercise. The animals were housed in appropriate conditions (21–22°C, 12 hour light/dark cycle, and free access to food and water) during the study period. The training tests were conducted in the light cycle. All the protocols followed the Consensus Author Guidelines on Animal Ethics and Welfare[12] and the national guidelines for conducting animal studies (Ethics Committee permission No. 94–19, Kerman University of Medical Sciences, Kerman, Iran). The animals were excluded from the study if they failed to do exercise training.

Surgical technique

The BFR groups were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and their hind leg skin was shaved and cleaned. Next, bilateral incisions were made to the inner surface of the animals' thighs to approach the femoral arteries. After dissection and exposure, each femoral artery (distal to the inguinal ligament) was tightly tied with a stainless steel wire, 0.014 inch in diameter, using 5-0 silk suture.[13] Afterwards, the wires were carefully removed and a small amount of penicillin powder was placed on the wounds.[13] The wounds were thereafter sutured and the animals were allowed to recover from anesthesia. In the Sh groups, the surgical method was the same as that in the BFR groups, but the femoral arteries were released without being tied. The recovery period

from surgery was 1 week. This model of femoral artery stenosis does not reduce the blood flow during muscle relaxation but it restricts the blood flow during activity or muscle contraction.[13] Limb blood perfusion after the partial obstruction was confirmed by measuring the blood flow in the rats' limbs with a Doppler laser device.

Training protocol

All the training groups were participated in a 10-week treadmill running program, 5 days a week. The program was commenced at 15 minutes per session at a speed of 7.5 meters per minute and at a slope of 0°. The speed of the treadmill and the duration of the exercise sessions were gradually increased until the final week, to 60 minutes per session at a speed of 15 meters per minute; however, the treadmill inclination was not changed. Additionally, as from the second week, the animals had 5 minutes of warm-up and 5 minutes of cool-down at a speed of 7 meters per minute.[14] The nontrained animals (groups without exercise) were familiarized with the silent treadmill for 20 minutes in each session.

Sampling and hypertrophy index measurement

Forty-eight hours after the last training session (in order to eliminate the effects of acute exercise), all the rats from all the groups were weighed and then sacrificed under deep anesthesia. Immediately after the killing, the soleus and the EDL were removed, cleaned from fat and connective tissues, weighed, and then frozen in liquid nitrogen. Thereafter, the muscles were preserved at -80°C up to the time of the analysis of the parameters. The soleus and the EDL were selected because the former is a postural muscle with a large number of slow-twitch motor units, while the latter contains a large number of fast-twitch motor units with less motor-unit recruitment in ordinary movements.[15] The muscle atrophy or muscle hypertrophy index[16] was calculated as the ratio of the muscle weight (mg) to the body weight (g).

Western blotting

The muscle samples were powdered with liquid nitrogen and lysed using a combination of a protease inhibitor (Sigma Aldrich Company, USA; S8820) and a radioimmunoprecipitation assay (RIPA) buffer (Sigma Aldrich Company, USA; R0278). For tissue homogenization, a homogenizer (Micro Smash TOMY, Japan) was utilized to perform 3 consecutive centrifugations at 3000 rpm for 5 minutes. The final products were centrifuged at 12 000 rpm for 20 minutes at 4°C. After the extraction of supernatants, the protein concentration of each sample was determined using the Bradford method (Bio-Rad Laboratories, Munich, Germany). The protein samples were mixed with a sample buffer (SDS, Glycerol, Tris, Bromophenol blue, 2-mercaptoethanol) before they were heated for 5 minutes at 95°C. Equal concentrations of the samples were loaded onto a 10% polyacrylamide gel and electrophoresed (100 V, 2 h). At the next step, the proteins were transferred on polyvinylidene difluoride membranes (Thermo Fisher Scientific Inc.; 88518), and the loaded membranes were incubated with a 5% blocking buffer (5 g of nonfat milk, 20 mM of Tris-buffered saline, and 0.1% Tween 20) for 2 hours. After the membranes were washed 3 times with TBST (Tris-buffered saline and Tween 20), the membrane-bound proteins were exposed to anti-AChR β2 antibody (1:1000; Sc-130936; Santa Cruz Biotechnology, USA) and anti-β-actin antibody (1:1000; Cell Signaling Technology, USA) as primary antibodies and goat anti-rabbit IgG-HRP antibody (1:10,000; Sc-2004; Santa Cruz Biotechnology, USA) as a secondary antibody for an overnight period and a 2-hour duration, respectively. An enhanced chemiluminescence reagent (Amersham ECL Prime Western Blotting Detection Reagent) and Lumi-Film chemiluminescent detection film (Roche) were used for the detection of immunoreactions, and VisionWorks®LS Analysis Software was employed to analyze the corresponding intensities. Anti β-actin antibody was used as a loading control.[17]

Statistical analysis

The data are provided as means ± standard errors (SEs). The statistical analyses were conducted with SPSS, version 20 (SPSS Inc., Chicago, Illinois, USA). The normal distribution of the data was confirmed by the Shapiro–Wilk test. Comparisons were performed between the different groups by one-way ANOVA and post hoc Tukey's test. A paired *t*-test was used for the comparison of pre- and post weights in each group. A P value<0.05 was considered statistically significant.

Results

Weight and hypertrophy indices

At the onset of the study, there was no significant difference in weight among the animals. At the end of the study period, in comparison with their primary weight, the CTL and Sh groups showed significant weight gain, the BFR group exhibited nonsignificant weight loss (1%), and the Ex, Sh+Ex, and BFR+Ex

groups had significant weight loss. The most weight loss was observed in the BFR+Ex group (8.6%, P<0.001 vs. all the other groups except the Ex and Sh+Ex groups) (table 1). The hypertrophy index of the soleus increased in all the trained groups, but it was significant only in the BFR+Ex group in comparison with the CTL, Sh, and BFR groups (P<0.001) (figure 1). On the other hand, BFR alone was associated with a rise in the hypertrophy index of the EDL (P=0.02 vs. the CTL and Sh groups). In addition, BFR+Ex induced additive effects on the hypertrophy index of the EDL (P<0.001 vs. the CTL, Sh, Ex, and Sh+Ex groups, and P=0.006 vs. the BFR group) (figure 2).

nAChRs alterations

BFR+Ex was associated with an increase in nAChRs in the soleus (P=0.02 vs. the CTL and Sh groups) (figure 3). In the EDL, the expression of nAChRs showed an incremental trend in the BFR and Ex groups; nonetheless, it reached the significance level only in the BFR+Ex group (P=0.008) in comparison with the CTL and Sh groups (figure 4).

Discussion

In the present study, we sought to determine whether low-intensity aerobic exercise along with lower-limb BFR could improve aging-induced muscle atrophy and nAChRs downregulation. Our results showed that this model of exercise (the KAATSU model) significantly upregulated nAChRs at the NMJ and induced hypertrophy in both EDL and soleus when compared with corresponding groups. Still, exercise individually was not able to enhance these parameters significantly.

Previous studies have revealed that the aging process is associated with the remodeling of NMJs and the dispersion of nAChR clusters.[7] The age-dependent dispersion of nAChR clusters is partly attributed to changes in cytosolic calcium homeostasis through alterations in dihydropyridine receptors and calcium pump proteins and increase in free cytosolic calcium. Increased cytosolic Ca^{2+} may activate Ca^{2+} dependent proteases such as calpain, which in turn can interact with the nAChR clustering protein rapsyn and disrupt the nAChRs complex.[18] In addition, mitochondria as important players in buffering cytosolic Ca^{2+}

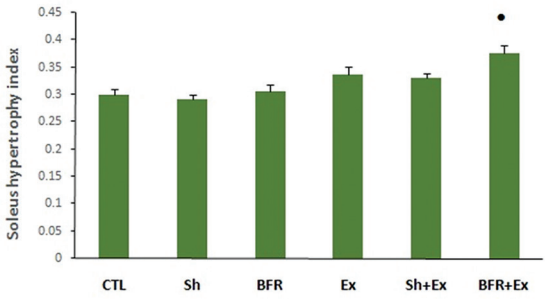

Figure 1: Soleus muscle hypertrophy index in the different experimental groups at the end of the study. Values are presented as means±SEMs. Numbers and abbreviations regarding the animal groups are presented in the legend to table 1. ●P<0.001 in comparison with the CTL, Sh, and BFR groups

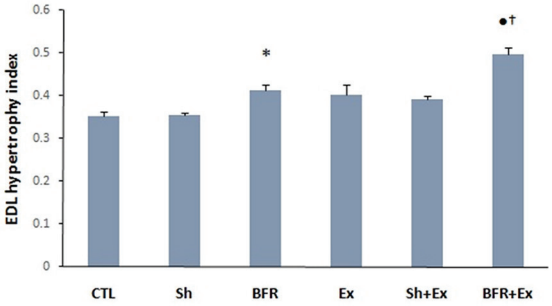

Figure 2: Extensor digitorum longus (EDL) muscle hypertrophy index in the different experimental groups at the end of the study. Values are presented as means±SEMs. Numbers and abbreviations regarding the animal groups are presented in the legend to table 1. *P=0.02 vs. the CTL and Sh groups; ●P<0.001 vs. the CTL, Sh, Ex, and Sh+Ex groups; †P=0.006 vs. the BFR group

Table 1: Data of the animals' weights in the different experimental groups

Groups/parameters	Pre weight	Post weight	P value (paired *t*- test)	ΔWeight%	P value (one-way ANOVA)
CTL	412±11	433±7▼	P=0.001	5.1±1.1	
Sh	417±14	438±13▼	P=0.001	5.1±0.8	
BFR	446±9	442±10	P=0.15	−1.04±0.6◦	P<0.001
Ex	410±12	386±10●	P<0.001	−5.9±0.5‡	P<0.001
Sh+Ex	406±11	390±9▼	P=0.001	−3.9±0. 5‡	P<0.001
BFR+Ex	439±10	401±10●	P<0.001	−8.7±1.2‡	P<0.001

Values are means±SEMs. CTL: Control; Sh: Sham animal group, which underwent surgery without blood-flow restriction; BFR: Animal group subjected to surgery and restriction of the hind blood flow during activity; Ex: Animal group subjected to prolonged low-intensity treadmill exercise; Sh+Ex: Animal group subjected to surgery without blood-flow restriction and then prolonged low-intensity treadmill exercise; BFR+Ex: Animal group subjected to blood-flow restriction and prolonged low-intensity treadmill exercise; ▼P=0.001 vs. pre weight in the same group; ● P<0.001 vs. pre weight in the same group; ‡P<0.001 vs. the CTL, Sh, and BFR groups; ◦ P<0.001 vs. the CTL and Sh groups

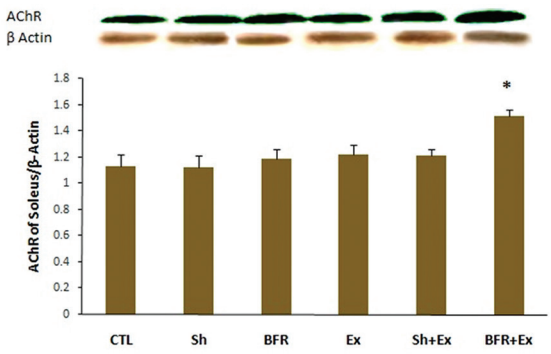

Figure 3: Shows expression of nicotinic acetylcholine receptors (AChRs) in the soleus muscle using the western blot method. Values are means±SEMs. Numbers and abbreviations regarding the animal groups are presented in the legend to Table 1. *P=0.02 vs. the CTL and Sh groups

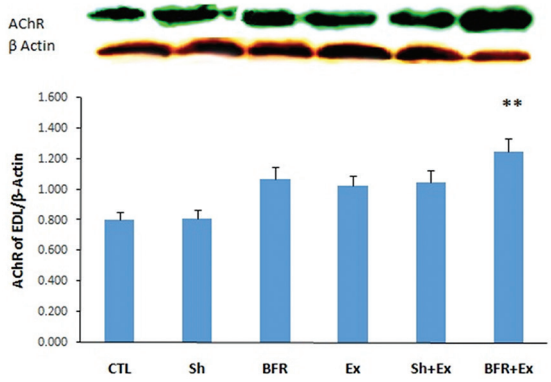

Figure 4: Shows expression of nicotinic acetylcholine receptors (AChRs) in the extensor digitorum longus (EDL) muscle using the western blot method. Values are means±SEMs. Numbers and abbreviations regarding the animal groups are presented in the legend to Table 1. **P=0.008 vs. the CTL and Sh groups

released by the sarcoplasmic reticulum may be depolarized when muscle fibers encounter calcium overload, which precedes significant neuromuscular degeneration.[2] It seems that in the wake of age-associated NMJ remodeling, NMJ responsiveness to exercise training is also modulated.[14] Indeed, unlike young rats, 10 weeks of low-intensity exercise in a previously conducted investigation failed to show remarkable effects on the NMJ and morphological structures of slow- and fast-twitch muscles in old rats.[14]

Chiming in with a study by Deschenes et al.,[14] we showed that exercise alone was unable to improve nAChR clustering at the NMJ in old rats. Nevertheless, 10 weeks of low-intensity running along with BFR upregulated nAChR clustering at the NMJ among these animals.

It is reported that the expression of genes implicated in mitochondrial energy metabolism is downregulated in sarcopenic rats with NMJ disruption.[19] Consequently, a decline in the number and function of mitochondria as well as frequent modifications of their morphological structure with aging may be a central factor in the reduction of NMJ responsiveness to exercise.[20] Nonetheless, in the current study, a combination of BFR and low-intensity exercise ameliorated the negative effects of aging on NMJ responsiveness to exercise and recovered the nAChR clusters in both soleus (slow-twitch) and EDL (fast-twitch). This finding supports the hypothesis that BFR when combined with exercise may be able to activate cellular signals within the nervous system and muscles and lead to changes in the NMJ. This cellular signaling induction may result from BFR-induced longer durations of muscle O_2 depletion and hypoxia in limbs during exercise.[21,22] Thus, anaerobic metabolism, increase in lactate, and decrease in blood pH occur.[21,22] Increase in mitochondrial production (e.g. in sub-sarcolemma mitochondria) can help cytosolic calcium regulation and its buffering and enhance the preservation and proliferation of nAChRs at the NMJ.[23] Moreover, lactic acidosis along with exercise may be associated with fatigue in traditional oxidative fibers (type I) but recruitment of glycolytic fibers.[24,25] This can lead to alterations in the phenotypes of glycolytic fibers and improvement in their nAChRs and NMJs. Other factors involved in nAChR clustering are nitric oxide (NO) and nitric oxide synthase (NOS).[26] NOS inhibition decreases agrin-induced nAChR clustering[27] and NO induction increases nAChRs at the NMJ.[28] Interestingly, the expression of neural nitric oxide synthase (nNOS) is decreased by aging and endurance exercise can upregulate its production in gastrocnemius and soleus muscles.[29] Accordingly, reinforcement of NO production in the presence of exercise and BFR may have been another mechanism behind the promotion of nAChR clustering at the NMJ in our study.

In the present study, we also found that 10 weeks of BFR along with mild exercise led to hypertrophy in the EDL and soleus muscles. This finding is in agreement with some previous studies which showed that 3 or 6 weeks of treadmill walk training plus BFR increased the mass and cross-sectional area of thigh muscles.[30] In this regard, although not everything is known about the molecular cell signaling that leads to muscle hypertrophy responses, some plausible mechanisms have been suggested. For example, BFR+Ex is associated with the production of some metabolites such as lactic acid, which causes muscle cell swelling. Cell swelling can be detected by intrinsic volume sensors, which results in the activation of these

sensors. This in turn may reduce proteolysis and activate the mammalian target of rapamycin (mTOR) and mitogen-activated protein-kinase (MAPK) pathways. Activation of these signaling pathways increases muscle protein synthesis and hence muscle hypertrophy.[31,32] In concordance with this theory, Fujita[22] demonstrated that a single bout of 20% of 1-repetition maximum-intensity knee-extension exercise with KAATSU enhanced the Akt/mTOR signaling pathway and thigh muscle protein synthesis in young men. In regular conditions, according to the size principle of multiple motor-unit recruitment, smaller motor units (slow-twitch type I) are employed first in low-intensity activities, while larger motor units (fast-twitch type Π) are recruited in higher-intensity levels of physical training.[33] Nevertheless, in BFR along with exercise, the contractive muscles encounter ischemia, hypoxia, and accumulation of metabolites in the tissue, all of which can affect their responsiveness during activity. This situation may stimulate the afferent nerves III and IV, which in turn leads to the recruitment of type Π muscle fibers.[24,25] Hence, in the BFR+Ex group, more employment and recruitment of glycolytic fibers due to ischemic environment-induced fatigue in traditional oxidative fibers[24,25] can explain the more prominent hypertrophy in the EDL, known as a fast-twitch muscle. In addition, aging is associated with more changes in the end-plate morphology and NMJ remodeling of fast motor units.[20,34] This may, therefore, increase the susceptibility of fast motor units to hypertrophy in response to BFR+Ex.

In the current study, the trained animals (the Ex, Sh+Ex, and BFR+Ex groups) showed weight loss at the end of the experiment. This finding is consistent with the results reported by Deschenes et al.[14] Previous studies have indicated that age-related alterations in body components result in an increase in fat and a decrease in muscle mass.[35,36] It is reported that in normal-weight individuals and in obese subjects, body weight tends to increase in the elderly.[37] On the other hand, exercise training increases the catabolism of fat and decreases the size of adipose tissue.[38] In addition, endurance training stimulates PGC1α and mitochondria production.[39] Reduction in adipose tissue and increase in mitochondria production can partly explain the weight loss in our trained animal groups. Due to the current dearth of information, however, the mechanism of the preventive effects of BFR on weight gain is unknown and as such needs supplementary studies.

Conclusion

Our results indicated that BFR along with low-intensity exercise conferred beneficial effects on muscle mass and nAChR clustering at the NMJ in old rats. Although more studies are warranted to extend this finding to humans, the emerging picture is that a combination of BFR and low-intensity exercise may be considered a safe method to improve muscle mass and motor skills among old persons.

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Conflict of Interest: None declared.

References

1. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol. 1998;147:755-63. doi: 10.1093/oxfordjournals.aje.a009520. PubMed PMID: 9554417.
2. Jang YC, Van Remmen H. Age-associated alterations of the neuromuscular junction. Exp Gerontol. 2011;46:193-8. doi: 10.1016/j.exger.2010.08.029. PubMed PMID: 20854887; PubMed Central PMCID: PMC3026920.
3. Deschenes MR. Motor unit and neuromuscular junction remodeling with aging. Curr Aging Sci. 2011;4:209-20. doi: 10.2174/1874609811104030209. PubMed PMID: 21529328.
4. American College of Sports Medicine Position Stand. The recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in healthy adults. Med Sci Sports Exerc. 1998;30:975-91. PubMed PMID: 9624661.
5. Deschenes MR, Tenny KA, Wilson MH. Increased and decreased activity elicits specific morphological adaptations of the neuromuscular junction. Neuroscience. 2006;137:1277-83. doi: 10.1016/j.neuroscience.2005.10.042. PubMed PMID: 16359818.
6. Arthur ST, Cooley ID. The effect of physiological stimuli on sarcopenia; impact of Notch and Wnt signaling on impaired aged skeletal muscle repair. Int J Biol Sci.

2012;8:731-60. doi: 10.7150/ijbs.4262. PubMed PMID: 22701343; PubMed Central PMCID: PMC3371570.
7. Sato Y. The history and future of KAATSU training. Int J Kaatsu Train Res. 2005;1:1-15. doi: 10.3806/ijktr.1.1.
8. Loenneke JP, Wilson JM, Marin PJ, Zourdos MC, Bemben MG. Low intensity blood flow restriction training: a meta-analysis. Eur J Appl Physiol. 2012;112:1849-59. doi: 10.1007/s00421-011-2167-x. PubMed PMID: 21922259.
9. Nakajima T, Yasuda T, Sato Y, Morita T, Yamasoba T. Effects of Exercise and Anti-Aging. ANTI-AGING MEDICINE. 2011;8:92-102. doi: 10.3793/jaam.8.92.
10. Gorgin Karaji Z, Gharakhanlou R, Rajabi S, Parnoo A. Effect of combination training on nicotinic acetylcholine receptors level in fast- and slow-twitch muscle threads of male Wistar rats. Quarterly of Horizon of Medical Sciences. 2013;18:165-70. [In Persian].
11. American College of Sports M. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. Med Sci Sports Exerc. 2009;41:687-708. doi: 10.1249/MSS.0b013e3181915670. PubMed PMID: 19204579.
12. The Guide for the Care and Use of Laboratory Animals. Ilar j. 2016;57:Np. doi: 10.1093/ilar/ilw049. PubMed PMID: 28053083.
13. Yang HT, Ogilvie RW, Terjung RL. Low-intensity training produces muscle adaptations in rats with femoral artery stenosis. J Appl Physiol (1985). 1991;71:1822-9. PubMed PMID: 1761479.
14. DDeschenes MR, Roby MA, Glass EK. Aging influences adaptations of the neuromuscular junction to endurance training. Neuroscience. 2011;190:56-66. doi: 10.1016/j.neuroscience.2011.05.070. PubMed PMID: 21683772; PubMed Central PMCID: PMC3156295.
15. Alshuaib WB, Fahim MA. Effect of exercise on physiological age-related change at mouse neuromuscular junctions. Neurobiol Aging. 1990;11:555-61. doi: 10.1016/0197-4580(90)90117-i. PubMed PMID: 2234287.
16. Binayi F, Joukar S, Najafipour H, Karimi A, Abdollahi F, Masumi Y. Erratum to: The Effects of Nandrolone Decanoate Along with Prolonged Low-Intensity Exercise on Susceptibility to Ventricular Arrhythmias. Cardiovasc Toxicol. 2015;15:290. doi: 10.1007/s12012-015-9320-4. PubMed PMID: 25846341.
17. Hajali V, Sheibani V, Mahani SE, Hajializadeh Z, Shabani M, Aliabadi HP, et al. Ovariectomy does not exacerbate the negative effects of sleep deprivation on synaptic plasticity in rats. Physiol Behav. 2015;144:73-81. doi: 10.1016/j.physbeh.2015.03.010. PubMed PMID: 25748255.
18. Renganathan M, Messi ML, Delbono O. Dihydropyridine receptor-ryanodine receptor uncoupling in aged skeletal muscle. J Membr Biol. 1997;157:247-53. doi: 10.1007/s002329900233. PubMed PMID: 9178612.
19. Ibebunjo C, Chick JM, Kendall T, Eash JK, Li C, Zhang Y, et al. Genomic and proteomic profiling reveals reduced mitochondrial function and disruption of the neuromuscular junction driving rat sarcopenia. Mol Cell Biol. 2013;33:194-212. doi: 10.1128/MCB.01036-12. PubMed PMID: 23109432; PubMed Central PMCID: PMC3554128.
20. Gonzalez-Freire M, de Cabo R, Studenski SA, Ferrucci L. The Neuromuscular Junction: Aging at the Crossroad between Nerves and Muscle. Front Aging Neurosci. 2014;6:208. doi: 10.3389/fnagi.2014.00208. PubMed PMID: 25157231; PubMed Central PMCID: PMCPmc4127816.
21. Abe T, Kearns CF, Sato Y. Muscle size and strength are increased following walk training with restricted venous blood flow from the leg muscle, Kaatsu-walk training. J Appl Physiol (1985). 2006;100:1460-6. doi: 10.1152/japplphysiol.01267.2005. PubMed PMID: 16339340.
22. Fujita S, Abe T, Drummond MJ, Cadenas JG, Dreyer HC, Sato Y, et al. Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis. J Appl Physiol (1985). 2007;103:903-10. doi: 10.1152/japplphysiol.00195.2007. PubMed PMID: 17569770.
23. Hardie DG, Hawley SA, Scott JW. AMP-activated protein kinase--development of the energy sensor concept. J Physiol. 2006;574:7-15. doi: 10.1113/jphysiol.2006.108944. PubMed PMID: 16644800; PubMed Central PMCID: PMC1817788.
24. Yasuda T, Abe T, Sato Y, Midorikawa T, Kearns CF, Inoue K, et al. Muscle fiber cross-sectional area is increased after two weeks of twice daily KAATSU-resistance training. International Journal of KAATSU Training Research. 2005;1:65-70. doi: 10.3806/ijktr.1.65.

25. Clark BC, Fernhall B, Ploutz-Snyder LL. Adaptations in human neuromuscular function following prolonged unweighting: I. Skeletal muscle contractile properties and applied ischemia efficacy. J Appl Physiol (1985). 2006;101:256-63. doi: 10.1152/japplphysiol.01402.2005. PubMed PMID: 16514004.
26. Jones MA, Werle MJ. Nitric oxide is a downstream mediator of agrin-induced acetylcholine receptor aggregation. Mol Cell Neurosci. 2000;16:649-60. doi: 10.1006/mcne.2000.0901. PubMed PMID: 11083925.
27. Godfrey EW, Schwarte RC. Nitric oxide and cyclic GMP regulate early events in agrin signaling in skeletal muscle cells. Exp Cell Res. 2010;316:1935-45. doi: 10.1016/j.yexcr.2010.03.016. PubMed PMID: 20346357.
28. Akimitsu T, Gute DC, Korthuis RJ. Leukocyte adhesion induced by inhibition of nitric oxide production in skeletal muscle. J Appl Physiol (1985). 1995;78:1725-32. PubMed PMID: 7544340.
29. Song W, Kwak HB, Kim JH, Lawler JM. Exercise training modulates the nitric oxide synthase profile in skeletal muscle from old rats. J Gerontol A Biol Sci Med Sci. 2009;64:540-9. doi: 10.1093/gerona/glp021. PubMed PMID: 19304939; PubMed Central PMCID: PMC2800810.
30. Abe T, Sakamaki M, Fujita S, Ozaki H, Sugaya M, Sato Y, et al. Effects of low-intensity walk training with restricted leg blood flow on muscle strength and aerobic capacity in older adults. J Geriatr Phys Ther. 2010;33:34-40. PubMed PMID: 20503732.
31. Berneis K, Ninnis R, Haussinger D, Keller U. Effects of hyper- and hypoosmolality on whole body protein and glucose kinetics in humans. Am J Physiol. 1999;276:E188-95. PubMed PMID: 9886966.
32. Loenneke JP, Fahs CA, Rossow LM, Sherk VD, Thiebaud RS, Abe T, et al. Effects of cuff width on arterial occlusion: implications for blood flow restricted exercise. Eur J Appl Physiol. 2012;112:2903-12. doi: 10.1007/s00421-011-2266-8. PubMed PMID: 22143843; PubMed Central PMCID: PMC4133131.
33. Clark BC, Manini TM, Hoffman RL, Williams PS, Guiler MK, Knutson MJ, et al. Relative safety of 4 weeks of blood flow-restricted resistance exercise in young, healthy adults. Scand J Med Sci Sports. 2011;21:653-62. doi: 10.1111/j.1600-0838.2010.01100.x. PubMed PMID: 21917016.
34. Kurokawa K, Mimori Y, Tanaka E, Kohriyama T, Nakamura S. Age-related change in peripheral nerve conduction: compound muscle action potential duration and dispersion. Gerontology. 1999;45:168-73. doi: 10.1159/000022081. PubMed PMID: 10202263.
35. Novak LP. Aging, total body potassium, fat-free mass, and cell mass in males and females between ages 18 and 85 years. J Gerontol. 1972;27:438-43. doi: 10.1093/geronj/27.4.438. PubMed PMID: 4627605.
37. Zamboni M, Mazzali G, Zoico E, Harris TB, Meigs JB, Di Francesco V, et al. Health consequences of obesity in the elderly: a review of four unresolved questions. Int J Obes (Lond). 2005;29:1011-29. doi: 10.1038/sj.ijo.0803005. PubMed PMID: 15925957.
38. Gollisch KS, Brandauer J, Jessen N, Toyoda T, Nayer A, Hirshman MF, et al. Effects of exercise training on subcutaneous and visceral adipose tissue in normal- and high-fat diet-fed rats. Am J Physiol Endocrinol Metab. 2009;297:E495-504. doi: 10.1152/ajpendo.90424.2008. PubMed PMID: 19491293; PubMed Central PMCID: PMC2724117.
39. Sutherland LN, Bomhof MR, Capozzi LC, Basaraba SA, Wright DC. Exercise and adrenaline increase PGC-1{alpha} mRNA expression in rat adipose tissue. J Physiol. 2009;587:1607-17. doi: 10.1113/jphysiol.2008.165464. PubMed PMID: 19221126; PubMed Central PMCID: PMC2678229.