ORIGINAL ARTICLE

Nr 2024;14 (3):396-401

The Impact of Vertical Jump Exercise in the Postsynaptic Cleft in Wistar Rats

Lara Caetano Rocha-Braga*, Isabella Gomes*, Jurandyr Pimentel Neto, André Neri Tomiate, Paula Oliveira Camargo, José Roberto Rodrigues da Silva, Adriano Polican Ciena

Laboratory of Morphology and Physical Activity (LAMAF), Institute of Biosciences, São Paulo State University (UNESP), Rio Claro-SP, Brazil

**The authors contributed equally to the work*

CORRESPONDING AUTHOR:
Adriano Polican Ciena
Laboratory of Morphology and Physical Activity (LAMAF)
Institute of Biosciences
São Paulo State University (UNESP)
Rio Claro 13506-900, Brazil
E-mail: adriano.ciena@unesp.br

DOI:
10.32098/mltj.03.2024.02

LEVEL OF EVIDENCE: 3

SUMMARY

Background. Skeletal muscle tissue demonstrates remarkable adaptability responding to stimuli at the neuromuscular junction (NMJ) during activities like vertical jumping, an essential ability in sports. Research about these changes can offer insights into neuromuscular plasticity.

Objective. The study aimed to explore the neuroplasticity of the postsynaptic cleft at the NMJ after vertical jump (VJ) training in twenty 90-day-old male Wistar rats, divided into Sedentary (S) and Jumper (J) groups.

Methods. A combination of immunostaining and microscopy was used to analyze structural and functional changes at the NMJ. α-Bungarotoxin highlighted the postsynaptic components, while fluorescence microscopy detailed the tissue and NMJ structures. Statistical analyses, including the Mann-Whitney test and the Shapiro-Wilk test, were used to compare groups and test data normality, with a significance level of $p < 0.05$ to ensure the relevance of the results.

Results. The morphometric analysis of the NMJ showed that the J group had a smaller postsynaptic cleft compared to the S group. There were no significant differences in dispersion, AChR area, endplate area, or endplate perimeter between the groups but lower values in J Group ($p < 0.05$). However, the AChR perimeter was significantly lower in the J group ($p < 0.05$). Additionally, the J group had fewer AChR clusters and a lower fragmentation index, both significantly different ($p < 0.005$).

Conclusions. VJ training modifies the NMJ affecting the organization and morphology of the motor endplate. This underscores the role of physical exercise in neuromuscular remodeling and calls for further studies to understand the functional implications of these adaptations.

KEY WORDS

α-bungarotoxin; exercise; neuromuscular junction; neuroplasticity vertical jump.

INTRODUCTION

The vertical jump (VJ) is an ability to generate force in a short period of time resulting in propulsion. The main active muscles in the execution of the VJ exercise are the lower limbs in humans (1). The VJ are also used for isoinertial field tests of neuromuscular function and response related to muscle fatigue (2). Additionally, the efficiency of the VJ is dependent on the properties of the muscle-tendon region, which is regulated by the central nervous system to ensure the appropriate muscular activation (3).

The neuromuscular junction (NMJ) is a chemical synapse region anatomically and functionally differentiated. The NMJ is composed mainly of axon terminal, muscle fiber, and terminal glial cells (*i.e.*, perisynaptic Schwann cells). The NMJ links the lower nervous system with the skeletal muscle fibers to induce muscle contraction (4).

The morphology of the postsynaptic cleft can change as a result of exercise (5), disuse (6), anabolic steroids (7), and according to the myofiber type (8). The synaptic activity conducted by NMJ is essential in sports practices and conditioned by the acetylcholine (ACh) vesicles secretion (9).
The connection promoted between the peripheral nerve and the muscle sarcolemma can be characterized by the signaling of the presynaptic and the postsynaptic cleft regions (10) (**figure 1**). In addition to the specialized action potential and consequent contraction, it is important to understand the state of synaptic activity, motor drive, denervation, ramification branches, and the different adaptation for the NMJ optimal performance.

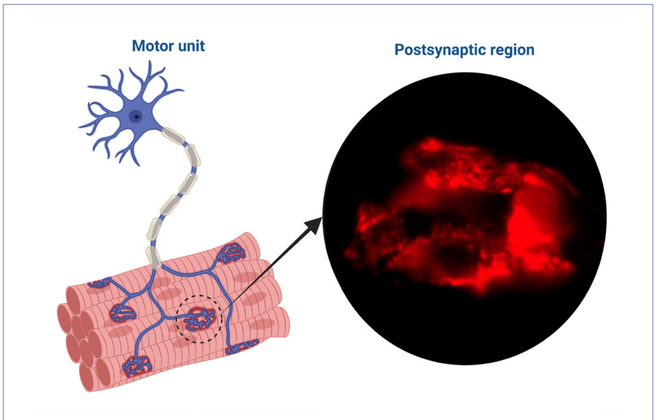

Figure 1. Illustration of the postsynaptic region and image of the NMJ receptors stained with α-bungarotoxin (created with BioRender.com).

Although athletes widely perform VJ to improve the muscle capacity of force production (11), most studies have only investigated the neuromuscular adaptations produced by traditional protocol training (*i.e.*, swimming, vertical ladder, stretching) (6-8).
The investigation of movements that involve concentric muscle contraction after an eccentric contraction is useful to understand the plasticity of the postsynaptic region (3). Therefore, this study aimed to investigate the morphological adaptations in the postsynaptic cleft of rats after VJ training.

MATERIALS AND METHODS

Animals

Twenty male Wistar rats 90 days old were divided into two groups (n = 10): Sedentary (S), not submitted to any protocol, and Jumper (J), submitted to VJ protocol (**figure 2**). The animals were kept in collective cages (n = 5) with food and water *ad libitum*, temperature (23 °C ± 2 °C) and photoperiod light/dark of 12h. All the procedures applied in this study were approved by the Ethical Committee of Animal Use (CEUA) of the Biosciences Institute - UNESP (Rio Claro, SP, Brazil; n° 1096/2021 – date of approval: May 12, 2021).

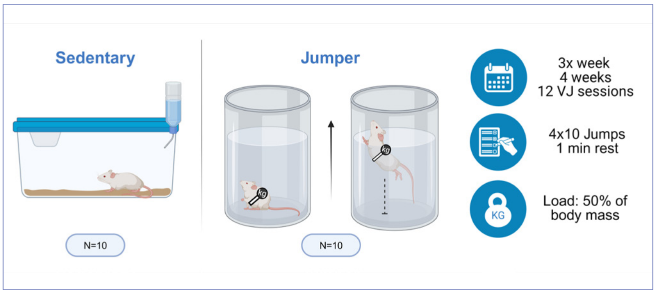

Figure 2. Illustration of the arrangement of experimental groups: Sedentary, Jumper and the characteristics of the protocol (created with BioRender.com).

Vertical jump

The VJ training was performed in cylindrical ducts, which were immersed in a rectangular tank filled with warm water (31 °C) at 30 cm depth. The animals of the J group performed 12 VJ sessions 3x/week over a four-week period. Each session was composed of 4 sets of 10 jumps interspersed with 1 min rest totalizing 40 jumps per session. In addition, an extra load of 50% of the animal's body mass was fixed to the chest to perform the VJ. Each vertical impulse performed by the animal was considered as one jump (12).

Sample collection and preparation

Five animals from each experimental group were euthanized with an anesthetic overdose (ketamine 100 mg/kg and xylazine 50 mg/kg) *i.p.* injection. The right gastrocnemius muscle was dissected, cryo-fixed in liquid nitrogen (-196 °C), kept in a biofreezer (-80 °C) and used for immunofluorescence protocol and analysis.

Immunofluorescence

For the immunofluorescence analysis the samples were cut into longitudinal sections (100 μm thick, Cryostat MICROM™ HM505E) prepared in slides and washed 2x/5 min with phosphate-buffered saline (PBS), permeabilized in 0.1% Triton X-100 solution (10 min), blocked in bovine serum albumin (BSA) 1% diluted in PBS (30 min) and incubated overnight (16h) with α-bungarotoxin anti-

body tetramethylrhodamine conjugate (BTX; Molecular Probes™, Eugene, OR-T-1175; 1:600). To establish the positioning of the NMJ in the muscle fiber the slides were incubated overnight (16h) with anti-myosin (skeletal slow) antibody (1:500 - M8421, Sigma-Aldrich®, OR, USA;) (**figure 3**). Following the rising of the slides with PBS 2x/5 min at 4 °C, the process was concluded by applying ProLong Gold™ (Molecular Probes™, Eugene, OR, USA) (13).
The images were obtained by 100X objective lens (plus 10X ocular magnification) through an Olympus BX61™ (Fully Motorized Fluorescence Microscope Shinjuku, Japan), equipped with a Fluorescence UIS2 optical system by Texas Red filter with the Software CellSens v.11.

Neuromuscular junction morphometry

The morphometry of the postsynaptic components was performed to analyze the motor endplate, adaptations and was established 10 images per animal. We measured the

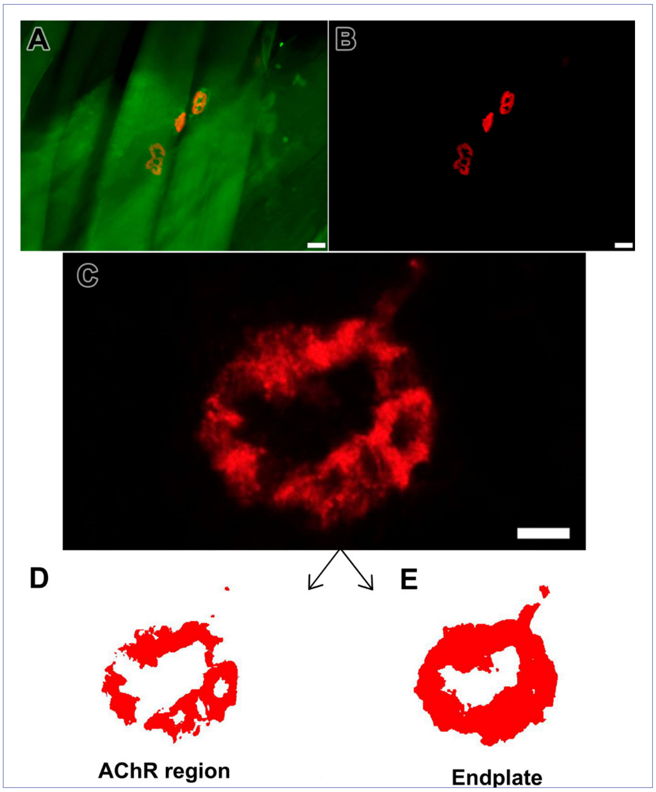

Figure 3. **(A)** Immunostaining image of α-bungarotoxin (red) and anti-myosin (green) to evidence the muscle fiber; **(B)** Immunostaining image of three endplates (red); **(C)** Immunofluorescence endplate image. Bars: 20 μm; **(D)** The C image binarized by ImageJ™ to evidence and mensure the AChR region variables; **(E)** The C image is binarized to evidence and measure the endplate variables.

following postsynaptic components (**figure 3**): endplate perimeter (motor endplate set outline), ACh receptors perimeter (stained receptor set outline), endplate total area (stained and unstained receptors set area), AChR area (stained receptor set area), and dispersion (AChR area/total endplate area × 100) (14). For the postsynaptic components identification were established the internal (AChR) and external (endplate) outline of the NMJ image by intensity of reddish color (6). For the average area, AChR clusters (μm^2) were calculated (AChR area/number of AChR clusters), and the fragmentation index was derived by Jones *et al.* (15) and Barbosa *et al.* (16).
All the measurements were performed by the ImageJ™ Software (NIH, Bethesda, MD, USA).

Statistical analysis

The obtained data were analyzed through Shapiro-Wilk normality test; all the variables were presented as non-parametric. This way, the statistical analysis performed were the unpaired t test with Welch's correction. The significance level established was $p < 0.05$. All statistical analyses were conducted using GraphPad Prism 8.4.3® software.

RESULTS

We reported the results of the VJ in an aquatic environment, and their effects on the morphology of the postsynaptic clefts.

Neuromuscular junction analysis

The morphometric analysis of the NMJ revealed a smaller postsynaptic cleft of J group rather than the S Group (**figure 4**), however, some structures present highlighted alterations. We observed lower values of dispersion (%) in the J group ($p > 0.05$), also the AChR area (μm^2), endplate area (μm^2), and endplate perimeter (μm) presented a decrease despite no significant statistical difference ($p > 0.05$), besides that, we observed lower and significant AChR perimeter (μm) ($p < 0.05$) in the J group. Furthermore, the J group demonstrated a lower number of AChR clusters (unit) ($p < 0.005$) and a lower fragmentation index ($p < 0.005$).

DISCUSSION

Our results reveal the adaptation due to VJ training. The main finding of the present study was the smaller postsynaptic components. The VJ is a functional capacity associated with acute and chronic neuromuscular fatigue following exercise (16).
Exercises such as resistance training can benefit from the VJ exercise modality as it holds the potential impact both efficacy and performance (17). The exercise practice also adapts the synaptic discharge pattern in the NMJ and consequently to its morphology (4).

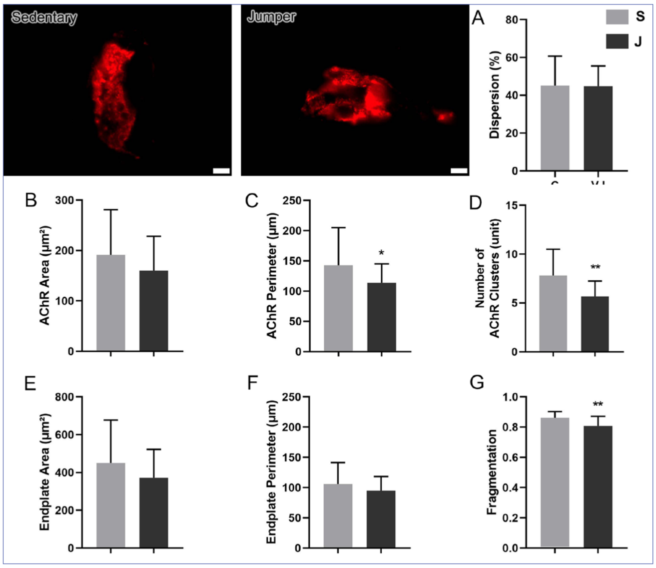

Figure 4. Endplate images of Sedentary (S) Group and Jumper (J) Group. Bars: 20 μm.

Mean and standard deviation of **(A)** dispersion (%); **(B)** AChR area (μm²); **(C)** AChR perimeter (μm) *J ≠ S, $p < 0.05$; **(D)** AChR clusters (unit) **J ≠ S, $p < 0.005$; **(E)** endplate area (μm²); **(F)** endplate perimeter (μm); and **(G)** of the endplate fragmentation index **J ≠ S, $p < 0.005$.

The data in the present study clearly show that the training period was possibly insufficient to make adaptations in muscle size, even the aquatic environment attenuated the effects that could normally occur during the stretch–shortening cycles. Cutlip *et al.* (18) reported increasing muscle mass in young rats during a 4.5-week period of stretching-shortening cycles exposure. In humans, there is a significant increase in muscle volume (19) after 4 and 8 weeks.

The VJ NMJ morphology presenta smaller AChR perimeter, number of clusters, and fragmentation after VJ training. Krause Neto *et al.* (7), reported in an experimental model of resistance training the increased motor endplate area. Besides, Pimentel Neto *et al.* (20) describes a decrease in the endplate area when analyzing rats' forelimbs subsequent to resistance training. Regardless, the aquatic environment requires riser muscle power for propulsion, resulting in high contractile intensities, and consequent muscle fatigue. This creates a unique response and adaptation in NMJ morphology to the aquatic environment. Fatigue induced by high contractile intensities in exercise can reduce NMJ dispersion, resulting in structural remodeling of the NMJ morphology (21, 22).

These alterations can be attributed to the adaptation of the molecular pathways associated with AChR such as agrin and MuSK proteins, noradrenaline, cAMP, and protein kinase A (13, 23, 24).

Different types of myofiber present different NMJ innervation, this results in morphological changes in the AChR clusters (25). So, the variation observed can be correlated with the increase in type II fibers.

Fragmentation demonstrates the redistribution of synaptic components over a larger area, in addition, it can also impact denervation and reinnervation and consequently act on neuromuscular transmission (26).

Our results demonstrated that the J Group presented a lower fragmentation and indicates the effect of fatigue in the synapse redistribution. Jones *et al.* (27) reported no evidence of age-associated change in the fragmentation of NMJ in humans. However, the reduction in NMJ fragmentation is directly associated with unloading in humans (28), in different neuropathies and denervation (29), and with the impairment in mTORC1 (30).

In summary, fragmentation is the result of an effective and normal process of NMJ plasticity, which enables the NMJ to adjust to changes in external conditions, allowing it to maintain its function (31).

Limitations of the study

The main limitations of the present study are the non-use of functional analysis regarding the proposed exercise protocol to understand the specific development of the Jumper group compared to the Sedentary. Furthermore, functional and molecular analyzes will be carried out regarding the neuromuscular signaling process, a factor that should also be considered as a limitation of this study.

CONCLUSIONS

We concluded that the present research revealed that vertical jump protocol induces adaptations in the morphology of the neuromuscular junction (NMJ), showing a reduction in postsynaptic components. Despite that, VJ in an aquatic environment attenuate the effects on stretching and shortening cycles. The lower fragmentation observed suggests a distinct response to fatigue in the VJ group, highlighting the adaptive capacity of the NMJ to specific training conditions. These findings highlight the uniqueness of the NMJ response to VJ and its ability to adjust in contexts of high contractile intensity.

FUNDINGS

This research was funded by São Paulo Research Foundation (FAPESP), grant number #2020/07418-4, and in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

LCRB, IG: writing – original draft. APC, IG: conceptualization. LCRB, IG, JRRS, JPN: methodology. LCRB, CSJ, IG: software. LCRB, CSJ, IG, JRRS, POC, JPN: formal analysis. APC: resources, supervision. APC, LCRB, IG, CSJ, JPN, POC, ANT: writing – review & editing. APC, LCRB, IG: funding acquisition.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests**.**

REFERENCES

1. Sattler T, Sekulic D, Hadzic V, Uljevic O, Dervisevic E. Vertical jumping tests in volleyball: reliability, validity, and playing-position specifics. J Strength Cond Res. 2012;26(6):1532-8. doi: 10.1519/JSC.0b013e318234e838.
2. Gathercole RJ, Sporer BC, Stellingwerff T, Sleivert GG. Comparison of the capacity of different jump and sprint field tests to detect neuromuscular fatigue. J Strength Cond Res. 2015;29(9):2522-31. doi: 10.1519/JSC.0000000000000912.
3. Taube W, Leukel C, Gollhofer A. How neurons make us jump: the neural control of stretch-shortening cycle movements. Exerc. Sport Sci Rev. 2012;40(2):106-15. doi: 10.1097/JES.0b013e31824138da.
4. Alvarez-Suarez,P, Gawor M, Prószyński TJ. Perisynaptic schwann cells - The multitasking cells at the developing neuromuscular junction. Semin Cell Dev Biol. 2020;104:31-8. doi: 10.1016/j.semcdb.2020.02.011.
5. Deschenes MR, Sherman EG, Roby MA, Glass EK, Harris MB. Effect of resistance training on neuromuscular junctions of young and aged muscles featuring different recruitment patterns. J Neurosci Res. 2015;93(3):504-13. doi: 10.1002/jnr.23495.
6. Rocha LC, Jacob CS, Barbosa GK, Pimentel Neto J, Krause Neto W, Gama EF, Ciena AP. Remodeling of the skeletal muscle and postsynaptic component after short-term joint immobilization and aquatic training. Histochem Cell Biol. 2020;154(6):621-8. doi: 10.1007/s00418-020-01910-9.
7. Krause Neto W, Silva WA, Ciena AP, Anaruma CA, Gama EF. Divergent effects of resistance training and anabolic steroid on the postsynaptic region of different skeletal muscles of aged rats. Exp Gerontol. 2017;98:80-90. doi: 10.1016/j.exger.2017.08.018.
8. Jacob CS, Barbosa GK, Rodrigues MP, Pimentel Neto J, Rocha-Braga LC, Ciena AP. Stretching prior to resistance training promotes adaptations on the postsynaptic region in different myofiber types. Eur J Histochem. 2022;66(1):3356. doi: 10.4081/ejh.2022.3356.
9. Nishimune H, Shigemoto K. Practical anatomy of the neuromuscular junction health and disease. Neurol Clin. 2018;36(2):231-40. doi: 10.1016/j.ncl.2018.01.009.
10. Cruz PMR, Cossins J, Beeson D, Vincent A. The neuromuscular junction in health and disease: molecular mechanisms governing synaptic formation and homeostasis. Front Mol Neurosci. 2020;13:10964. doi: 10.3389/fnmol.2020.610964.
11. Ramirez-Campillo R, Garcia-Hermoso A, Moran J, Chaabene H, Negra Y, Scanlan AT. The effects of plyometric jump training on physical fitness attributes in basketball players: a meta-analysis. J Sport Health Sci. 2020;11(6):656-70. doi: 10.1016/j.jshs.2020.12.005.
12. Malanotte JA, Kakihata CMM, Karvat J, Brancalhão RMC, Ribeiro LFC, Bertolini GRF. Jumping in aquatic environment after sciatic nerve compression: nociceptive evaluation and morphological characteristics of the soleus muscle of Wistar rats. Einstein. 2017;15(1):77-84. doi: 10.1590/S1679-45082017AO3613.
13. Deschenes MR, Kressin KA, Garratt RN, Leathrum CM, Shaffrey EC. Effects of exercise training on neuromuscular junction morphology and pre- to post-synaptic coupling in young and aged rats. Neuroscience. 2016;316:167-77. doi: 10.1016/j.neuroscience.2015.12.004.
14. Jones RA, Reich CD, Dissanayake KN, et al. NMJ-morph reveals principal components of synaptic morphology influencing structure - function relationships at the neuromuscular junction. Open Biol. 2017;6(12):160240. doi: 10.1098/rsob.160240.
15. Barbosa GK, Jacob CS, Rodrigues MP, Rocha LC, Pimentel Neto J, Ciena AP. Morphological changes in the motor endplate and in the belly muscle induced by previous static stretching to the climbing protocol. Microsc Microanal. 2021;27:1183-91. doi: 10.1017/S1431927621012253.
16. Alexiou H, Coutts AJ. A comparison of methods used for quantifying internal training load in women soccer players. Int J Sports Physiol Perform. 2008;3(3):320-30. doi: 10.1123/ijspp.3.3.320.
17. Watkins CM, Barillas SR, Wong MA, et al. Determination of vertical jump as a measure of neuromuscular readiness and fatigue. J Strength Cond Res. 2017;31(12):3305-10. doi: 10.1519/JSC.0000000000002231.
18. Cutlip RG, Baker BA, Geronilla KB, et al. Chronic exposure to stretch–shortening contractions results in skeletal muscle adaptation in young rats and maladaptation in old rats. Appl Physiol Nutr Metab. 2006;31(5):573-87. doi: 10.1139/h06-033.
19. Kubo K, Morimoto M, Komuro T, Yata H, Tsunoda N, Kanehisa H, Fukunaga T. Effects of plyometric and weight training on muscle–tendon complex and jump performance. Med Sci Sports Exerc. 2007;39(10):1801-10. doi: 10.1249/mss.0b013e31813e630a.
20. Pimentel Neto J, Rocha LC, Jacob CS, Barbosa GK, Ciena AP. Postsynaptic cleft density changes with combined exercise protocols in an experimental model of muscular hypertrophy. Eur J Histochem. 2021;35(s1):3274. doi: 10.4081/ejh.2021.3274.
21. Deschenes MR, Covault J, Kraemer WJ, Maresh CM. The neuromuscular junction: muscle fibre type differences, plas-

ticity and adaptability to increased and decreased activity. Sports Med. 1994;17(6):358-72. doi: 10.2165/00007256-199417060-00003.
22. Shembel AC, Lenell C, Chen S, Johnson AM. Effects of vocal training on thyroarytenoid muscle neuromuscular junctions and myofibers in young and older rats. J. Gerontol. A Biol Sci Med Sci. 2021;76(2):244-52. doi: 10.1093/gerona/glaa173.
23. Madhavan R, Peng HB. Molecular regulation of postsynaptic differentiation at the neuromuscular junction. IUBMB Life. 2005;57(11):719-30. doi: 10.1080/15216540500338739.
24. Bukharaeva EA, Samigullin D, Nikolsky E, Vyskocil F. Protein kinase A cascade regulates quantal release dispersion at frog muscle endplate. J Physiol. 2002;538(Pt 3):837-48. doi: 10.1113/jphysiol.2001.012752.
25. Zhao Y, Peng HB. Roles of tyrosine kinases and phosphatases in the formation and dispersal of acetylcholine receptor clusters. Neurosci Lett. 2020;733:135054. doi: 10.1016/j.neulet.2020.135054.
26. Zamparo P, Minetti AE Prampero PE. Interplay among the changes of muscle strength, cross-sectional area and maximal explosive power: Theory and facts. Eur J Appl Physiol. 2002;88(3):193-202. doi: 10.1007/s00421-002-0691-4.
27. Jones RA, Harrison C, Eaton SL, Hurtado ML, et al. Cellular and molecular anatomy of the human neuromuscular junction. Cell Rep. 2017;21(9):2348-56. doi: 10.1016/j.celrep.2017.11.008.
28. Monti E, Reggiani C, Franchi MV, et al. Neuromuscular junction instability and altered intracellular calcium handling as early determinants of force loss during unloading in humans. J Physiol. 2021;599(12):3037-61. doi: 10.1113/JP281365.
29. Iyer SR, Shah SB, Lovering RM. The neuromuscular junction: roles in aging and neuromuscular disease. Int J Mol Sci. 2021;22(15):8058. doi: 10.3390/ijms22158058.
30. Baraldo M, Geremia A, Pirazzini M, et al. Skeletal muscle mTORC1 regulates neuromuscular junction stability. J Cachexia Sarcopenia Muscle. 2020;11(1):208-25. 10.1002/jcsm.12496.
31. Slater CR. 'Fragmentation' of NMJs: a sign of degeneration or regeneration? A long journey with many junctions. Neuroscience. 2020;439:28-40. doi: 10.1016/j.neuroscience.2019.05.017.