

Relationship between calcium circulation-related factors and muscle strength in rat sciatic nerve injury model

Xiaoming Sun¹, Wei Wang¹, Yangyi Dong¹, Yue Wang¹, Meixiang Zhang¹, Zhao Wang¹, Xiaowei Yu¹, Jiao Huang¹, Hongxing Cai^{1*}

¹ Department of Forensic Medicine, Xuzhou Medical University, Xuzhou, Jiangsu, P. R. China

ARTICLE INFO

Article type:
Original article

Article history:
Received: Jun 10, 2019
Accepted: Dec 1, 2019

Keywords:
Forensic medicine
Muscle atrophy
Muscle strength
Ryanodine receptor
Sciatic nerve

ABSTRACT

Objective(s): The purpose of this study is to investigate the indication function of the calcium circulation-related factors on the damage to muscle strength and contraction function after nerve injury. The target factors include ryanodine receptor (RyR), inositol-1,4,5-triphosphate receptor (IP3R), phospholamban (PLN), cryptocalcitolin (CASQ), ATPase and troponin C (TNNC).

Materials and Methods: Sprague-Dawley (SD) rats were randomly divided into sham-operated group (SO), sciatic nerve injury group (SNI) and sciatic nerve disconnection group (SNT). Sciatic nerve function index and stretching test were used to examine the changes to muscle strength; bilateral gastrocnemius muscles were extracted after execution for gastrocnemius wet weight ratio test. HE staining slides and average cross-sectional area of muscle fibers were acquired to analyze the muscle atrophy. The transcription level of the factors was also measured.

Results: Sciatic nerve damage in SNI group was significantly higher than that in SO group in the 6 weeks, but there was no significant difference between SNT and SO groups following sciatic nerve damage. Sciatic nerve function in SNT group was worse than that in SNI group. The average cross-sectional area of gastrocnemius muscle fibers in SNI and SNT groups was significantly reduced compared to that in SO group. The transcriptional levels of RyR, PLN, CASQ, ATPase and TNNC in SNI and SNT groups were significantly different from those in SO group.

Conclusion: Calcium circulation-related factors could be used as potential indicators for assessment of damages to muscle strength.

► Please cite this article as:

Sun X, Wang W, Dong Y, Wang Y, Zhang M, Wang ZH, Yu X, Huang J, Cai H. Relationship between calcium circulation-related factors and muscle strength in rat sciatic nerve injury model. Iran J Basic Med Sci 2020; 23:654-662. doi: 10.22038/IJBMS.2020.40915.9695

Introduction

Injury of the motor system is a common type of injuries in the practice of forensic clinical identification. It is more common in sports injuries such as contusion, excessive stretching, tearing caused by accidents, muscle damage caused by surgery, etc. (1). Such injuries often impair the function of motor system, resulting in reduced muscle strength. Muscle strength refers to the strength, amplitude and speed of muscle contraction during active exercise. It is an important indicator for evaluating the body's ability to exercise (2). The initiation and regulation of muscle strength is a complex but coordinated biological process (3) involving the direct or indirect involvement of many related genes and proteins, especially the calcium related ones. At present, the inspection methods used still rely mainly on the active cooperation of the subjects to assess their muscle strength (4,5), which leads to poor reproducibility of inspection results and lack of objective inspection indicators. Therefore, the search for indicators that can be used to objectively quantify muscle strength has become one of the urgent needs of forensic clinical identification work and clinical evaluation of muscle strength.

The basic structural unit of skeletal muscle is muscle fiber, a slender and multinuclear structure composed of multiple myoblasts. A plurality of muscle fibers surrounded by connective tissue forms a muscle bundle,

and a plurality of muscle bundles forms the whole piece of muscle (6,7).

When a pulse from the nerve reaches the skeletal muscle cells, the calcium channel on the terminal cell membrane is opened and the calcium diffuses into the sarcoplasm. Calcium ions entering the sarcoplasm bind to troponin C (TNNC), causing changes in the conformation of TNNC and detachment of actin. The myosin head then combines with actin. With the participation of ATP, the head of myosin oscillates along the long axis of the filament, which causes actin to slide along the long axis of the filament, causing myofibril shortened and resulting in muscle contraction (8). At the end of the contraction, the Ca^{2+} - Mg^{2+} -ATPase on the sarcoplasmic reticulum reaggregates calcium ions in the cytosol into the terminal pool (9). Ryanodine receptor (RyR), inositol 1,4,5-triphosphate receptor (IP₃R) and phospholamban (PLN) are involved in the release of calcium ions from the sarcoplasmic reticulum; calsequestrin (CASQ) and ATPase are involved in the recovery and elimination of extracellular calcium after contraction; TNNC binds to calcium ions and participates in myofilament sliding and contraction. We investigated the transcriptional level of those calcium-related factors after nerve injury for the indication effect for muscle strength damage, to search precise quantitative indicators of muscle strength assessment for forensic clinical identification. Meanwhile, we also explored the effects of muscle

*Corresponding author: Hongxing Cai. Department of Forensic Medicine, Xuzhou Medical University, Xuzhou, Jiangsu, P. R. China. Tel: 0516-85748442 Email: yccaih1962@126.com

Table 1. Primer sequence used for RTPCR

Gene	Forward primer	Reverse primer
RyR1	AGAGGCTCCTGTACCAGCAG	AGGATGGAGATGCCAGCTT
PLN	AAGCCAAGGCTCCTAAAAG	TGATAGCCGAGCGAGTAAGG
CASQ1	GTCAATGTTACGGATGCGGAC	CTCCTTTTCTCCAGCGAGGG
ATP2a1	CCCAGATGGGGAAGGTCTA	TGCAATGTTGGTACCCGAGA
TNNC2	CCGCATCTTTGACAGGAACG	CCCAGAAGCCCGAAAATCT
IP ₃ R1	GTATTCACGAAAGCATTCTCC	CTTTTGTTCCTCCAAATCGCTG
GAPDH	GACATGCCGCTGGAGAAAC	AGCCCAGGATGCCCTTAGT

atrophy on muscle strength after detecting muscle damage at the molecular level.

Materials and Methods

Animal model for nerve injury

All experimental procedures were conducted in accordance with the Guidelines for Animal Experimentation of XuZhou Medical University and approved by the Committee for Animal Experimentation. One hundred 250 g male SD rats (SPF grade) were obtained from the animal center of XuZhou medical university. All rats were given free access to water and standard diet throughout the study and were maintained in a temperature-controlled (21-26 °C) and humidity-controlled (50-60%) room with a 12 hr light/dark cycle. The rats were divided at random into 3 groups (n=30): sham operation (SO) group, sciatic nerve injury (SNI) group, and sciatic nerve transection (SNT) group. All experimental rats were anesthetized deeply by intraperitoneal administration of 0.2 ml/100 g 2% sodium pentobarbital. The skeletal muscles of left lower extremity were separated to expose the sciatic nerve. No further surgical operations were performed in SO group. The left sciatic nerve was crushed for 30 sec with an 18 cm forceps hemostatic in SNI group and cut off in SNT group. Five rats from each group were executed by carbon dioxide on 3 days, 1, 2, 4, 6 and 8 weeks after nerve lesion, respectively. Whole gastrocnemius muscles were excised from left and right hind limbs and measured. The ratio of the left gastrocnemius muscle weight to the right gastrocnemius muscle weight was calculated. Samples were quick-frozen in liquid nitrogen for histological analysis and RT-qPCR testing.

Assessment of animal models

The extensor posture thrust (EPT) was applied as a reflection of muscle strength of lower limbs in small animals (10). The rat was placed on the operating floor and the upper part of the body was grasped in a disinfect towels. Only the hindlimbs were exposed. One hindlimb was supported with the experimenter's palm and the

other hindlimb was placed upon a digital calculation balance. The flat surface of the scale is covered with a piece of rough absorbent paper. NEPT and EEPT represent extensor postural thrust on the normal and experimental sides. The formula for calculating the functional deficit ratio is: functional deficit ratio=(NEPT-EEPT)/NEPT.

The sciatic nerve function index (SFI) is the current standard for measuring functional recovery following rat sciatic nerve injury. The rat's hindlimbs were dipped in a methylrosanilinium chloride solution, and it was permitted to walk down the track, which was covered with a strip of white paper. Measurements included print length on both the experimental and the normal sides (EPL, NPL), toe spread between the first and fifth digits on both sides (ETS, NTS), and the distance between the middle of the second and fourth toes on both sides (EIT, NIT). The formula for calculating the SFI is: $SFI = 109.5 (ETS-NTS)/NTS - 38.3 (EPL-NPL)/NPL + 13.3 (EIT-NIT)/NIT - 8.8$.

Consecutive gastrocnemius muscle sections were prepared in 5 µm thick sections. Histological examination of muscle fiber atrophy was assessed using hematoxylin and eosin (H&E) staining. The cross-sectional areas of the muscle fibers were measured with Image-Pro Plus, and at least 100 fibers per sample were counted (magnification×400).

RT-qPCR

Total RNA was extracted from the samples using TRNzol Reagent (DP405, TIANGEN) following the manufacturer's protocol. Subsequently, the concentration (ng/ml) and purity of the total RNA were evaluated using the multimode reader. Only RNAs with OD₂₆₀/OD₂₈₀ ratios fell in 1.8-2.0 were used in subsequent experiments.

The first strand cDNA was synthesized with PrimeScript™ RT Master Mix (KB118, TIANGEN), following the manufacturer's protocol. For the reverse transcription, 2 µg of total RNA was used in a final reaction volume of 20 µl.

Primers were designed and synthesized by Sangon Biotech (Shanghai)(Table 1). A 20 µl reaction mixture

was prepared for real-time PCR amplification with SuperReal PreMix Plus (FP205, TIANGEN), containing 10 μ l SuperReal PreMix Plus, 6.4 μ l RNase-free ddH₂O, 0.4 μ l each of the forward and reverse primers of genes of interest and the reference gene, 0.4 μ l 50 \times ROX Reference Dye, and 2.0 μ l cDNA. The reaction was performed with an Applied Biosystems 7500, with one round predenaturation at 95 °C for 15 min, and 40 rounds of denaturation at 95 °C for 10 sec followed by annealing and extension at 60 °C for 32 sec. Triple fluorescent signals (FAM, Cy5, and HEX or ROX) were measured synchronously at the end of each cycle. Fluorescence curves of the PCR products were evaluated using 7500 Software v2.0.5.

Statistical analyses

All results were expressed as mean \pm standard error (SE). The statistical difference was determined by one-way ANOVA. All statistical analyses were performed using SPSS, version 16.0, with P -value<0.05 considered statistically significant.

Results

After the establishment of animal models, the gastrocnemius muscle atrophy of SNI and SNT groups was different from that of SO group. From week 4 to week 8, the gastrocnemius muscle atrophy of SNI group was significantly lighter than that of SNT group (P -value<0.05). The wet weight ratios of gastrocnemius in SNI and SNT groups decreased significantly in the first two weeks (P -value<0.05), but in the fourth week, the wet weight ratio of gastrocnemius in SNI group increased significantly compared to that in the second week (P -value<0.05), and then continued to show an upward trend. The wet weight ratio of gastrocnemius in SNT group did not change significantly from week 4 to week 8 (P -value>0.05) (Figure 1).

The SFI of SNI group was not significantly different from that of SO group from week 6 (P -value>0.05).

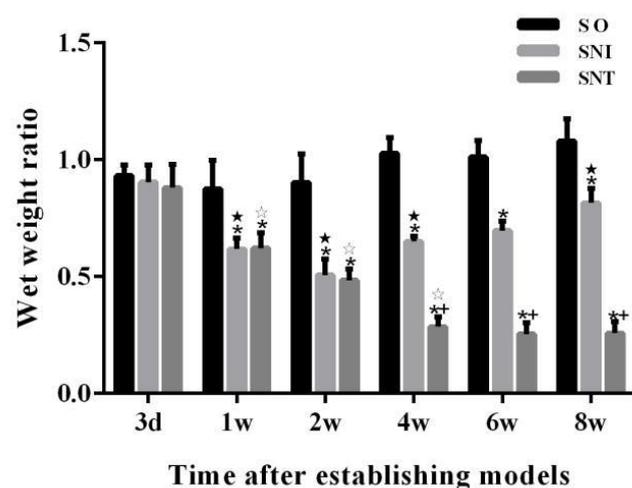


Figure 1. Gastrocnemius muscle wet weight ratio of rats
* vs SO group at the same time P -value<0.05. + vs SNI group at the same time P -value<0.05. ☆ vs SNI group at the previous time P -value<0.05. ☆+ vs SNT group at the previous time P -value<0.05
SO: Sham-operated group, SNI: Sciatic nerve injury group, SNT: Sciatic nerve disconnection group

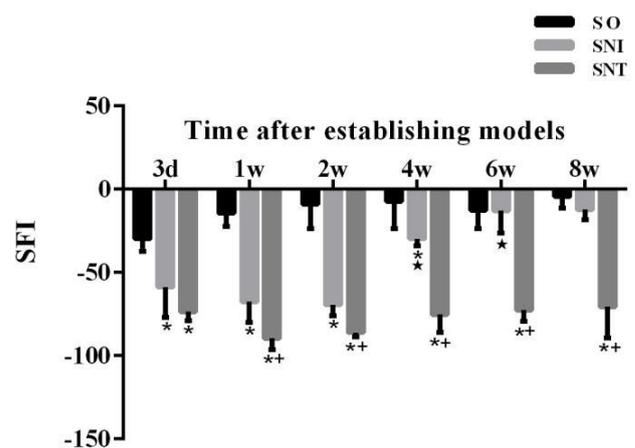


Figure 2. Sciatic nerve function index of rats
* vs SO group at the same time P -value<0.05. + vs SNI group at the same time P -value<0.05. ☆ vs SNI group at the previous time P -value<0.05.
SO: Sham-operated group, SNI: Sciatic nerve injury group, SNT: Sciatic nerve disconnection group

And from week 2 to week 8, the sciatic nerve function of SNI group was less damaged than that of SNT group (P -value<0.05). From week 4, the sciatic nerve function of SNI group was better than that in the previous time point (P -value<0.05); while in SNT group, the sciatic nerve was always in a state of serious injury without significant change (P -value>0.05) (Figure 2).

After the operation, the lower limb function of the operated side was impaired in all rats. The functional deficit ratios of SNI and SNT groups were significantly higher than that of SO group (P -value<0.05). The functional deficit ratio of SNT group was different from that of SNI group from week 1 to week 8 (P -value<0.05). In SNI group, the lower limb function of the operative side improved significantly compared to the previous time point from week 6 (P -value<0.05); in SNT group, the lower limb function of the operative side was severely impaired without change (P -value>0.05) (Figure 3).

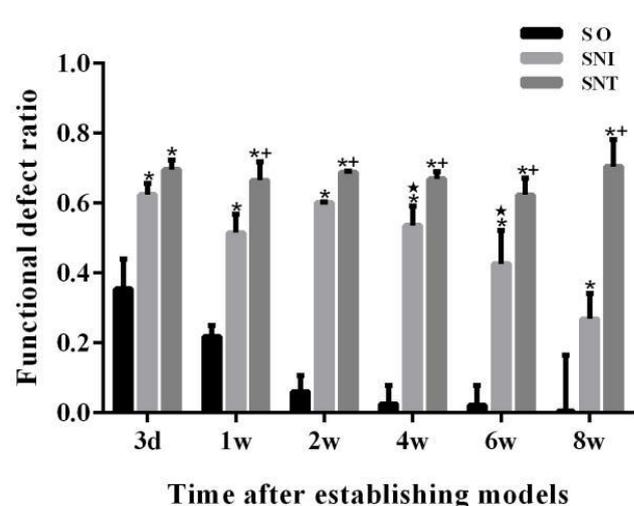


Figure 3. Functional defect ratio of rats
* vs SO group at the same time P -value<0.05. + vs SNI group at the same time P -value<0.05. ☆ vs SNI group at the previous time P -value<0.05.
SO: Sham-operated group, SNI: Sciatic nerve injury group, SNT: Sciatic nerve disconnection group

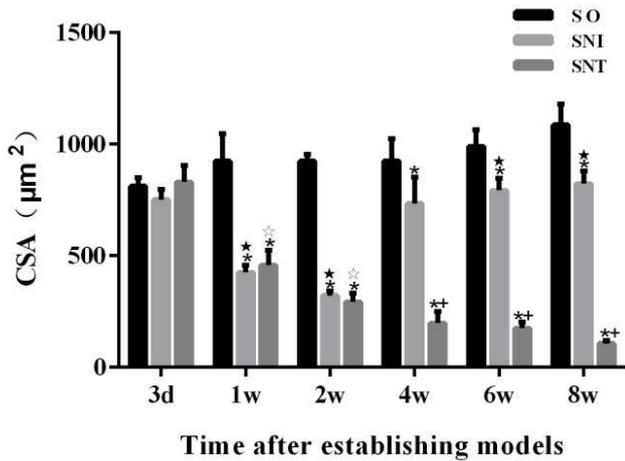


Figure 4. Gastrocnemius muscle fiber cross-sectional area of rats * vs SO group at the same time P -value<0.05. +vs SNI group at the same time P -value<0.05. ★ vs SNI group at the previous time P -value<0.05. ☆ vs SNT group at the previous time P -value<0.05 SO: Sham-operated group, SNI: Sciatic nerve injury group, SNT: Sciatic nerve disconnection group

There was no significant change in the average area of gastrocnemius muscle fibers in each group on the 3rd day after modeling (P -value>0.05). The cross-sectional areas of muscle fibers in SNI and SNT groups were smaller than that in SO group at the same time point from week 1 (P -value<0.05), but increased significantly in SNI group at the 4th week (P <0.05). The cross-sectional area of muscle fibers in SNI group decreased in the first two weeks (P -value<0.05), but increased significantly at week 4 compared to the previous time point (P -value<0.05), while the cross-sectional area of muscle fibers in SNT group decreased continuously (Figure 4, 5).

The transcription level of RyR in SNI group was significantly higher than that in SO group at week 1 and

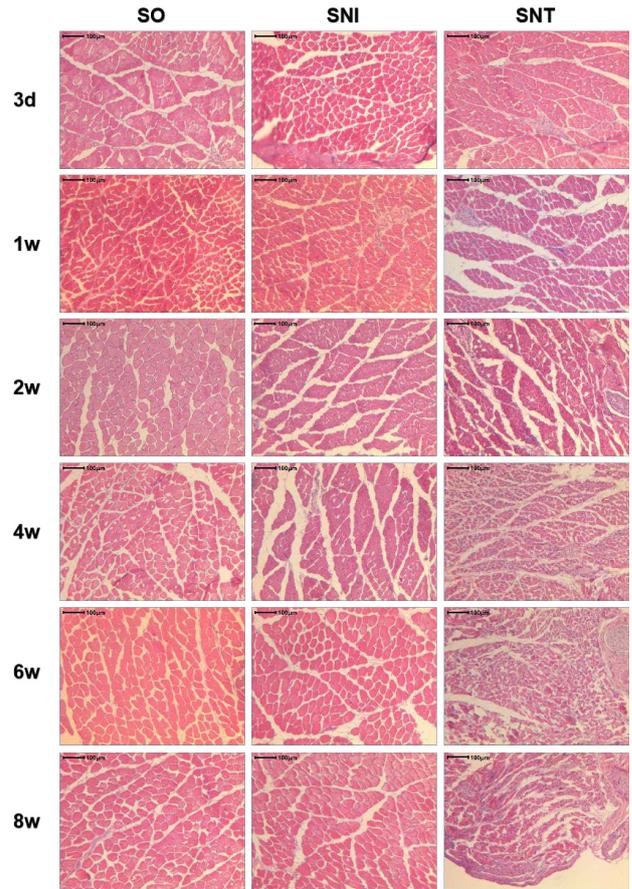


Figure 5. HE staining of gastrocnemius muscle cross section on the operative side of rats (100 times)

decreased from week 4 to week 8 (P -value<0.05); the transcription level of RyR in SNT group was significantly higher than that in SO group at week 1, week 2, week 4 and week 8 (P -value<0.05) (Figure 6).

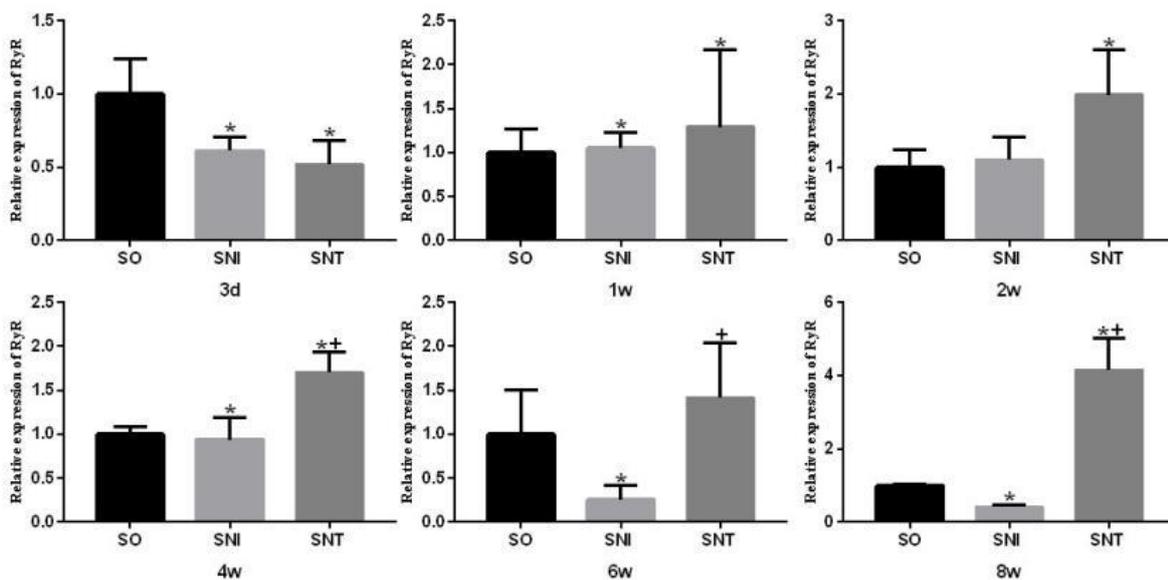


Figure 6. Transcription level of RyR1 in rat gastrocnemius muscle * vs SO group P -value<0.05. + vs SNI group P -value<0.05 SO: Sham-operated group, SNI: Sciatic nerve injury group, SNT: Sciatic nerve disconnection group, RyR: Ryanodine receptor

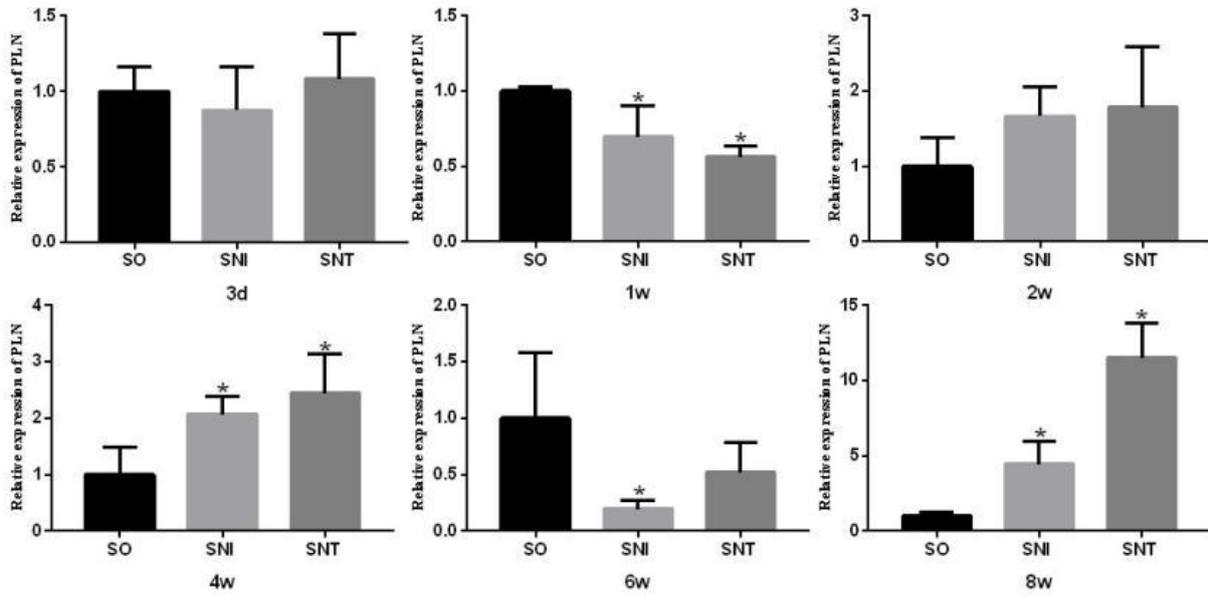


Figure 7. Transcription level of PLN in rat gastrocnemius muscle
 * vs SO group P -value<0.05
 SO: Sham-operated group, SNI: Sciatic nerve injury group, SNT: Sciatic nerve disconnection group, PLN: Phospholamban

Compared to SO group, the transcription levels of PLN in SNI and SNT groups decreased significantly at week 1 and week 6 (P -value<0.05), and increased at week 2, 4 and 8, especially at week 4 and week 8 (P -value<0.05) (Figure 7).

The transcription level of CASQ in SNI group was lower than that in SO group, which was more significant on the 3rd, 1st, 4th and 6th weeks (P -value<0.05). And that in SNT group was significantly lower than that in SO group only at the first week (P -value<0.05). There was no significant difference between SO group and SNT group at the other time points (P -value>0.05) (Figure 8).

The transcription level of ATPase in SNI group was lower than that in SO group at week 2 (P -value<0.05); the transcription level of ATPase in SNT group was lower than that in SO group at the third day and the second week (P -value<0.05), and there was no significant change at other time points (P -value>0.05) (Figure 9).

The transcription level of TNNC in SNI group was lower than that in SO group at week 1 and 6 (P <0.05), while that was lower at week 1 in SNT group, but significantly higher than that in SO group at week 4 (P <0.05) (Figure 10).

The changes of transcription level of RyR and TNNC

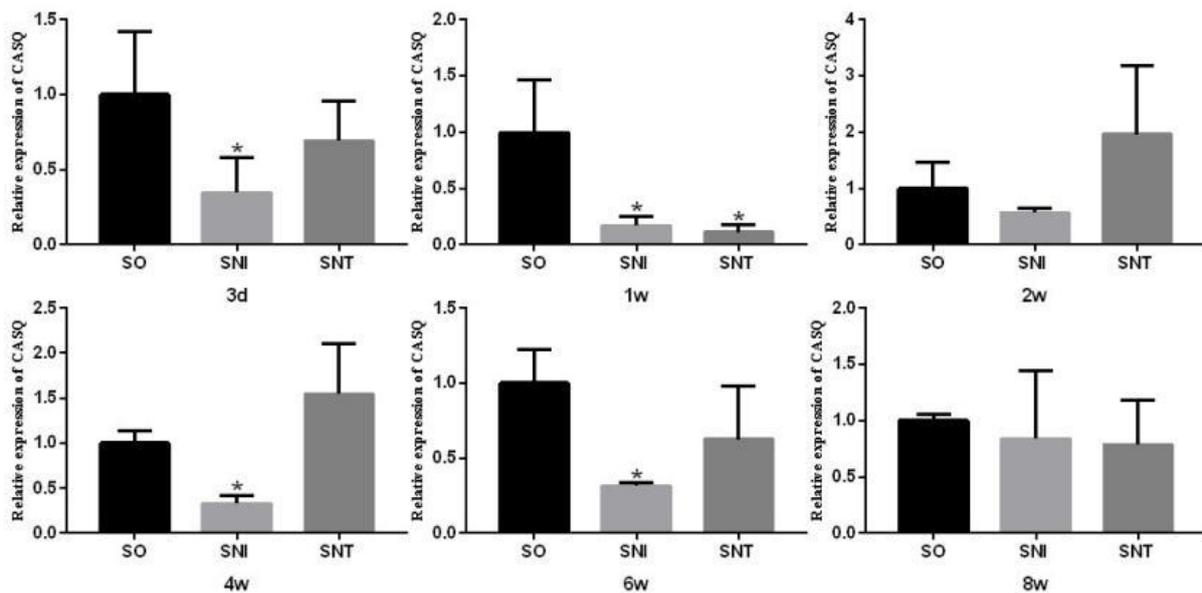


Figure 8. Transcription level of CASQ in rat gastrocnemius muscle
 *vs SO group P -value<0.05. + vs SNI group P -value<0.05
 SO: Sham-operated group, SNI: Sciatic nerve injury group, SNT: Sciatic nerve disconnection group, CASQ: Cryptocalcitonin

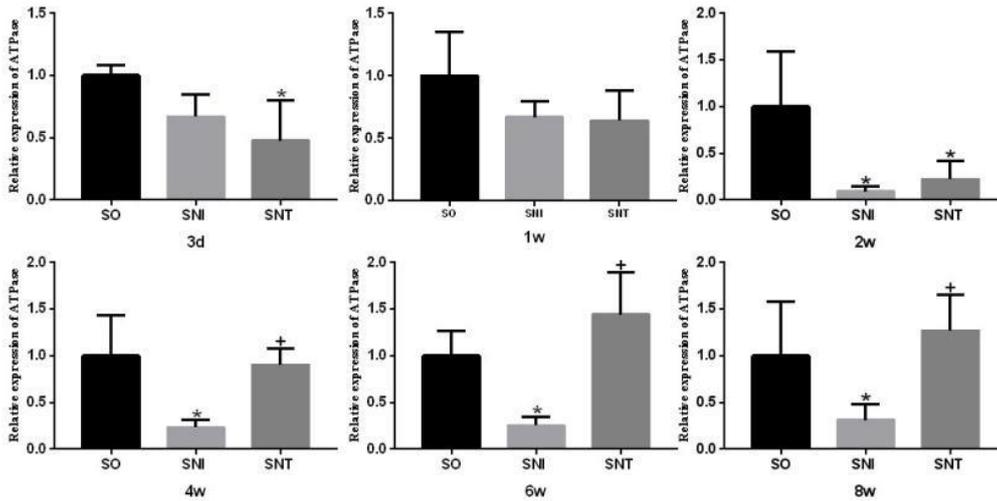


Figure 9. Transcription level of ATPase in rat gastrocnemius muscle
 *vs SO group P -value<0.05. + vs SNI group P -value<0.05
 SO: Sham-operated group, SNI: Sciatic nerve injury group, SNT: Sciatic nerve disconnection group

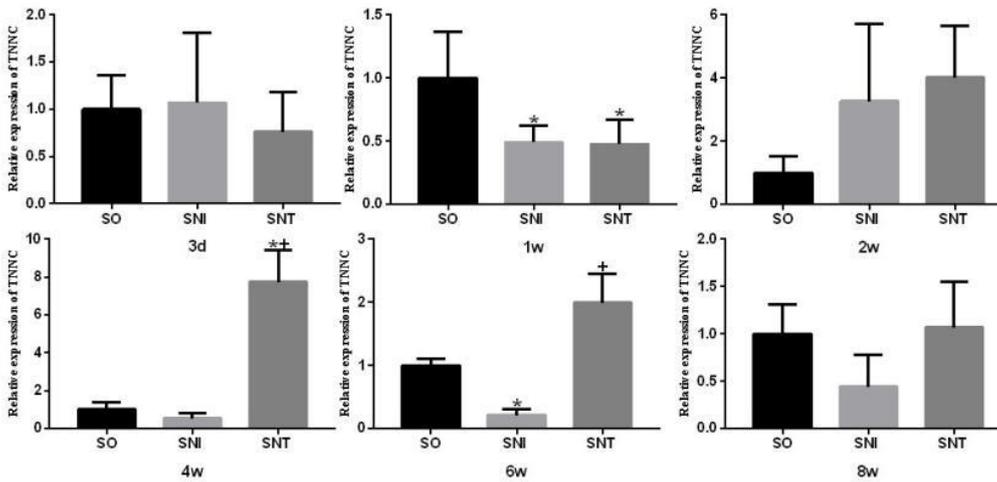


Figure 10. Transcription level of TNNC in rat gastrocnemius muscle
 *vs SO group P -value<0.05. + vs SNI group P -value<0.05
 SO: Sham-operated group, SNI: Sciatic nerve injury group, SNT: Sciatic nerve disconnection group, TNNC: Troponin C

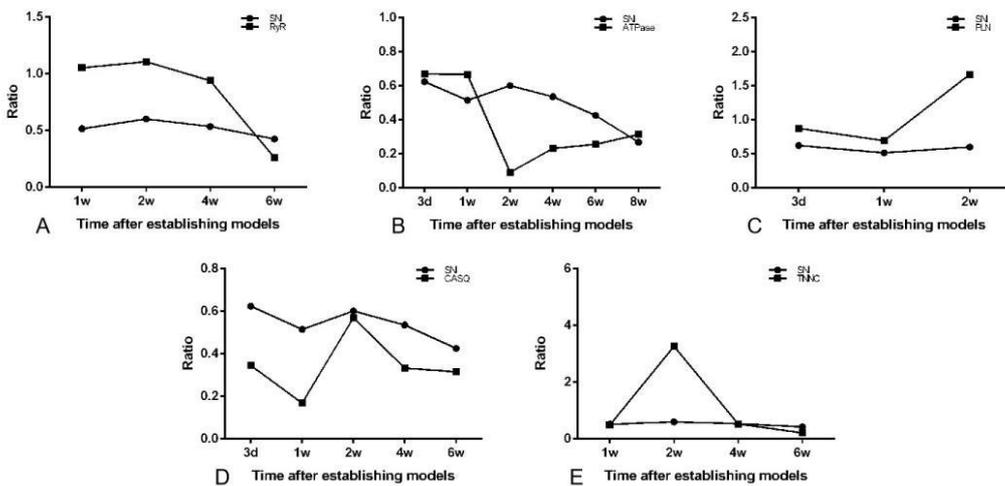


Figure 11. The transcription level of calcium cycle related genes in rat gastrocnemius muscle and the lower limb muscle function ratio in SNI group
 SNI: Sciatic nerve injury group

were opposite to those of muscle strength from week 1 to week 6; the changes of transcription level of PLN were opposite to those of muscle strength from day 3 to week 2, and the changes of transcription level of CASQ were opposite to those of muscle strength from day 3 to week 6. Also, the changes of transcription level of ATPase were the same as that of muscle strength throughout the experiment (Figure 11).

Discussion

At present, identification of limb muscle strength in forensic clinical work still relies mainly on unarmed muscle strength identification method, which has the shortcomings of over subjective and poor repeatability. Therefore, we hope to find target genes that are related to the muscle strength changes caused by nerve injury muscular atrophy, and to explore an objective and reproducible method for muscle strength measurement.

The basic structural unit of skeletal muscle is muscle fiber, which is a slender multi-nucleated mechanism formed by the fusion of myoblasts. Skeletal muscle fibers are mainly divided into slow muscle fibers consisting of type I fibers and fast muscle fibers consisting of type IIA, type IIX and type IIB fibers. In addition, there are a few intermediate phenotypes of different fiber types due to fiber mixing. Rat's gastrocnemius muscle is composed of slow and fast muscle fibers, and the proportion of the two kinds of muscle fibers is basically the same (11). The contractility of skeletal muscle is also closely related to the type of muscle fibers (12). After denervation, atrophic skeletal muscles exhibit malnutrition, accompanied by changes in fibers (13). In skeletal muscular atrophy, skeletal muscle fibers will gradually transform from type I to type II. Researchers have studied this phenomenon and found that type II muscle fibers are more likely to be dominated during the reinnervation of peripheral nerves after denervation, which may be a reason for muscle fiber conversion. It has been reported that the loss of muscle strength caused by nerve injury is greater than that observed in muscle atrophy (14). We hope to find a way to objectively express the changes of muscle strength in neuropathic muscular atrophy through this experiment. Therefore, we not only determine the degree of muscle atrophy through morphological experiments but also detect the changes of muscle strength of lower limbs in rats while maintaining a unified standard.

In this experiment, we found that the muscle loss in the SNI group was more severe than the muscle atrophy. This may be because the generation of muscle strength involves a variety of complex and coordinated biological processes (15) and does not depend entirely on muscle mass. Moreover, the excitatory-contraction coupling of skeletal muscle causes muscle contraction through calcium-mediated nerve stimulation, a process that is crucial for muscle strength production in skeletal muscle (16). Therefore, we studied the related changes of calcium cycle-related factors in the SNI model.

RyR is located in the sarcoplasmic reticulum terminal pool. There are three subtypes of these receptors including RyR1 (skeletal muscle), RyR2 (myocardium) and RyR3 (widely distributed, mainly in the brain). RyR1 is distributed in type I and type II muscle fibers. However,

the distribution density of RyR1 in type II muscle fibers is higher than that of type I muscle fibers (17). RyR is an important component of the excitatory-contraction coupling process (18). After nerve stimulation, calcium ions in the sarcoplasmic reticulum can be released by RyR1 (19), and damage to this mechanism will lead to muscle contraction disorders. It has been reported that the expression of RyR is related to the muscle state. The transcription level of RyR1 changes with the degree of muscle atrophy after denervation of skeletal muscle in rats. There is a significant increase in RyR1 mRNA expression in the soleus muscle composed of typical slow muscle fibers after long-term denervation (20). However, the results of RyR transcript levels obtained in experiments with other types of muscle atrophy leading to decreased muscle strength are not same. Findings show that RyR expression may decrease (21), or may not change depending on situation (22). In this experiment, after significant muscle atrophy in the rat model, the transcriptional level of RyR1, which expressed superiority in fast muscle fibers, was significantly elevated. After muscle recovery in muscle atrophy rats, fast muscle fibers can be converted into slow muscle fibers (23). When the muscle mass of the SNI group recovered, the transcription level of RyR1 was significantly decreased, which may be related to this phenomenon. When the muscle strength of rats in the SNI group changed significantly in the late stage of the experiment, the transcription level of RyR1 was different from that of the other two groups of rats whose muscle strength was intact and muscle strength was lost. From week 1 to week 6, the trend of RyR1 transcription was contrary to that of muscle strength, which might be used for the muscle strength test at the molecular level.

PLN is a 52-amino-acid integral membrane phosphoprotein, which regulates the intracellular calcium concentration by regulating the activity of Ca^{2+} -ATPase on the sarcoplasmic reticulum. Phosphorylated PLN increases the uptake of calcium ions in the sarcoplasmic reticulum and enhances the diastolic function of the muscle. PLN is present on the sarcoplasmic reticulum of the myocardium, slow contractile muscles and rapidly contracting muscles. Ca^{2+} -ATPase on the sarcoplasmic reticulum is also known as sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA). SERCA is a major regulator of free calcium ions in skeletal muscle cells. It can use the energy released by ATP hydrolysis to recover free calcium ions from the sarcoplasmic reticulum into the sarcoplasmic reticulum (24). In mammals, SERCA is mainly divided into three gene categories of SERCA1 (ATP2a1), SERCA2 (ATP2a2) and SERCA3 (ATP2a3), and can produce more than 10 subtypes (25). SERCA1 and SERCA2 subtypes are dominant in adult skeletal muscle; PLN and SERCA2 are expressed mainly in slow muscle fibers, and SERCA1 is mainly expressed in fast muscle fibers (26, 27). PLN regulates SERCA1 and SERCA2 by interacting with sarcolipin (SLN) to reduce the affinity of calcium ions on the sarcoplasmic reticulum surface (28). Studies have shown that after denervation, calcium concentration of the skeletal muscle sarcoplasmic reticulum decreases. PLN expression is significantly increased, but SERCA activity is halved (29). In the early stage of experiment,

the relative gene transcription level of PLN was opposite to that of muscle strength. The transcription level of ATP2a1 in the whole experiment was the same as that of muscle strength. This change in expression helps us to discover changes in muscle strength.

CASQ is the main binding protein for sarcoplasmic reticulum regulation of calcium storage, and it is mainly divided into CASQ1 and CASQ2 in mammals (30). In skeletal muscle, both CASQ1 and CASQ2 are expressed during development. CASQ2 is also abundantly expressed in adult slow muscle fibers, but CASQ2 expressed in fast muscle fibers is gradually replaced by CASQ1 after birth, and finally CASQ1 becomes the only CASQ subtype expressed by fast muscle fibers (31). Active calcium transport is limited by the concentration of free calcium ions in the sarcoplasmic reticulum (32). Fast muscle fibers release and absorb more calcium ions than slow muscle fibers. CASQ1 makes the free calcium ion concentration relatively low for SERCA more effective in the inward movement of calcium ions, which is essential for the maintenance of normal physiological activity of fast muscle fibers (33). It has been found that changes in muscle fiber types have a significant effect on calcium regulation in skeletal muscle during early denervation. The expression of CASQ1 with calcium storage function is slightly decreased (34), which is consistent with the change of transcriptional level of CASQ1 found in our early experiments. From the 3rd day to the 6th week, the transcription level of CASQ1 was contrary to the trend of muscle strength change, and it is expected to be used to detect muscle strength change.

TNNC is a complex composed of three subunits, troponin C, troponin I and troponin T. It is an important regulator of skeletal muscle contraction (35). It is divided into TNNC1 expressed in slow muscle fibers, TNNC2 expressed in fast muscle fibers and TNNC3 expressed in myocardium (36). Studies have shown that calcium ion in sarcoplasmic reticulum increases after denervation (37), and denervated skeletal muscle may increase the affinity of contractile proteins to calcium ions by increasing the expression of TNNC (38). This is consistent with the changes in the transcriptional level of TNNC2 found in the later period of our experiment. During the experiment, the transcriptional level of TNNC2 was opposite to that of muscle strength of rats in the SNI group.

Muscle strength changes in the SNI group can be attributed to complex interactions between multiple factors, such as signal transmission between nerves and muscles (39), muscle state (40) and excitation contraction coupling (41). In this experiment, we found that five calcium cycle-related genes have obvious expression changes in the SNI group, and have different expressions under different muscle strength states, and the transcriptional level of the gene and the trend of muscle strength are related in a certain period of time. However, the transcription level of housekeeping genes varies at different developmental stages, cell types, and experimental conditions (42). The transcriptional level of housekeeping genes in skeletal muscle is also affected by the sciatic nerve injury (43). In this experiment, we screened the housekeeping genes such as GAPDH, β -actin, Tbp, rpl32 and rps18 as internal parameters.

Finally, combined with experimental results and previous experimental studies, GAPDH and rps18 were selected as double internal reference genes. Though there is no optimal internal reference gene, the relative quantitative transcriptional level of the calcium cycle-related genes is still different at different muscle strength levels in this experiment. This suggests that we can establish a new detection system to determine muscle strength by quantitative analysis of the expression of different genes.

Conclusion

The transcriptional level of calcium circulation-related genes, such as RyR1, PLN, CASQ and TNNC, changed with the time elapse after nerve injury in rat. They could be potential useful molecular markers for precise assessment of muscle strength after nerve injury applied in forensic clinical work.

Acknowledgment

This work was supported by the Xuzhou Medical University Scientific Research Fund for Talents (D2016005 and D2016004)

The results described in this paper were part of student thesis.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

- Enger M, Skjaker SA, Nordsletten L, Pripp AH, Melhuus K, Moosmayer S, Brox JL. Sports-related acute shoulder injuries in an urban population. *BMJ Open Sport Exerc Med* 2019; 5: e000551.
- Steinbacher P, Eckl P. Impact of oxidative stress on exercising skeletal muscle. *Biomolecules* 2015; 5: 356-377.
- Miller MS, Bedrin NG, Ades PA, Palmer M, Toth MJ. Molecular determinants of force production in human skeletal muscle fibers: effects of myosin isoform expression and cross-sectional area. *Am J Physiol Cell Physiol* 2015; 308: C473.
- Setiyowati YD, Wang ST, Chen HM. Thermotherapy combined with therapeutic exercise improves muscle strength and depression in patients with ischemic stroke. *Rehabil Nurs* 2019;44:254-262.
- Mentiplay BF, Tan D, Williams G, Adair B, Pua YH, Bower KJ, et al. Assessment of isometric muscle strength and rate of torque development with hand-held dynamometry: Test-retest reliability and relationship with gait velocity after stroke. *Journal of biomechanics* 2018; 75: 171-175.
- Frontera W R, Ochala J. Skeletal Muscle: A brief review of structure and function. *Calcif Tissue Int* 2015; 96: 183-195.
- Berry DB, Regner B, Galinsky V, Ward SR, Frank LR. Relationships between tissue microstructure and the diffusion tensor in simulated skeletal muscle. *Magn Reson Med* 2018; 80:317-329.
- Gong H, Liu L, Jiang CL. Progress in the study of C2C12 cell contraction model and its contractile function. *Chin J Sport Med* 2015; 34: 78-81.
- Nozdrenko DM, Miroshnychenko MS, Soroca VM, Korchinska LV, Zavodovskiy DO. The effect of chlorpyrifos upon ATPase activity of sarcoplasmic reticulum and biomechanics of skeletal muscle contraction. *Ukr Biochem J* 2016;88:82-88.
- Emril DR, Wibowo S, Meliala L, Susilowati R. Cytidine 5'-diphosphocholine administration prevents peripheral neuropathic pain after sciatic nerve crush injury in rats. *J Pain Res* 2016;9:287-291.

11. Wiberg R, Jonsson S, Novikova LN, Kingham PJ. Investigation of the expression of myogenic transcription factors, microRNAs and muscle-specific E3 ubiquitin ligases in the medial gastrocnemius and soleus muscles following peripheral nerve injury. *PLoS One* 2015;10:e0142699.
12. Kaczmarek D, Łochyński D, Everaert I, Pawlak M, Derave W, Celichowski J. Role of histidyl dipeptides in contractile function of fast and slow motor units in rat skeletal muscle. *J Appl Physiol* (1985) 2016;121:164-172.
13. Romero NB. Centronuclear myopathies: a widening concept. *Neuromuscular Disorders Nmd* 2010; 20: 223-228.
14. Béatrice Chabi, Ljubovic V, Menzies KJ, Huang, JH, Saleem A, Hood DA. Mitochondrial function and apoptotic susceptibility in aging skeletal muscle. *Aging Cell* 2007; 7: 2-12.
15. Rassier, Dilson E. Sarcomere mechanics in striated muscles: from molecules to sarcomeres to cells. *American journal of physiology. Cell physiology* 2017; 313: C134-C145.
16. Järvillehto Matti, Mänttari Satu. Comparative analysis of mouse skeletal muscle fibre type composition and contractile responses to calcium channel blocker. *BMC Physiology* 2005; 5: 4.
17. Jayasinghe ID, Munro M, Baddeley D, Launikonis BS, Soeller C. Observation of the molecular organization of calcium release sites in fast- and slow-twitch skeletal muscle with nanoscale imaging. *J R Soc Interface* 2014;11: pii: 20140570.
18. Barone V, Randazzo D, Del Re V, Sorrentino V, Rossi D. Organization of junctional sarcoplasmic reticulum proteins in skeletal muscle fibers. *J Muscle Res Cell Motil* 2015; 36: 501-515.
19. Allard B. From excitation to intracellular Ca²⁺ movements in skeletal muscle: Basic aspects and related clinical disorders. *Neuromuscul Disord* 2018; 28: 394-401.
20. Díaz-Vegas AR, Cordova A, Valladares D, Llanos P, Hidalgo C, Gherardi G, *et al.* Mitochondrial calcium increase induced by RyR1 and IP3R channel activation after membrane depolarization regulates skeletal muscle metabolism. *Front Physiol* 2018;9:791.
21. Renganathan M. Caloric restriction prevents age-related decline in skeletal muscle dihydropyridine receptor and ryanodine receptor expression. *FEBS letters* 1998; 434: 346-350.
22. Thomas MM, Vigna C, Betik AC, Tupling AR, Hepple RT. Initiating treadmill training in late middle age offers modest adaptations in Ca²⁺ handling but enhances oxidative damage in senescent rat skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 2010; 298: R1269.
23. Green HJ, Klug GA, Reichmann H, Seedorf U, Wiehrer W, Pette D. Exercise-induced fibre type transitions with regard to myosin, parvalbumin, and sarcoplasmic reticulum in muscles of the rat. *Pflügers Arch* 1984; 400: 432-438.
24. Periasamy M, Maurya SK, Sahoo SK, Singh S, Sahoo SK, Reis FCG, Bal NC. Role of SERCA pump in muscle thermogenesis and metabolism. *Compr Physiol* 2017;7:879-890.
25. Hamm NC, Stammers AN, Susser SE, Hlynsky MW, Kimber DE, Kehler DS, *et al.* The regulation of sarco(endo)plasmic reticulum calcium-ATPases (SERCA). *Can J Physiol Pharmacol* 2015; 93: 843-854.
26. Periasamy M, Maurya SK, Sahoo SK, Singh S, Reis FCG, Bal NC. Role of SERCA pump in muscle thermogenesis and metabolism. *Compr Physiol* 2017; 7: 879-890.
27. Fajardo VA, Eric B, Chris V, Tahira D, Darin B, Daniel G, *et al.* Co-expression of SERCA isoforms, phospholamban and sarcolipin in human skeletal muscle fibers. *PLoS ONE* 2013; 8: e84304.
28. Asahi M, Kurzydowski K, Tada M, MacLennan DH. Sarcolipin inhibits polymerization of phospholamban to induce superinhibition of Sarco(endo)plasmic reticulum Ca²⁺-ATPases (SERCAs). *Journal of Biological Chemistry* 2002; 277: 26725-26728.
29. Komatsu M, Nakada T, Kawaqishi H, Kato H, Yamada M. Increase in phospholamban content in mouse skeletal muscle after denervation. *J Muscle Res Cell Motil* 2018; 39: 163-173.
30. Lewis KM, Munske GR, Byrd SS, Kang J, Cho HJ, Ríos E, *et al.* Characterization of post-translational modifications to calsequestrins of cardiac and skeletal muscle. *Int J Mol Sci* 2016;17: pii: E1539.
31. Boncompagni S, Protasi F, Franzini-Armstrong C. Sequential stages in the age-dependent gradual formation and accumulation of tubular aggregates in fast twitch muscle fibers: SERCA and calsequestrin involvement. *Age (Dordr)* 2012;34:27-41.
32. Shen X, Cannell MB, Ward ML. Effect of SR load and pH regulatory mechanisms on stretch-dependent Ca(2+) entry during the slow force response. *J Mol Cell Cardiol* 2013; 63:37-46.
33. Paolini C, Quarta M, D'Onofrio L, Reggiani C, Protasi F. Differential effect of calsequestrin ablation on structure and function of fast and slow skeletal muscle fibers. *J Biomed Biotechnol* 2011;2011:634075.
34. Hunter RB, Mitchell-Felton H, Essig DA, Kandarian, SC. Expression of endoplasmic reticulum stress proteins during skeletal muscle disuse atrophy. *AJP Cell Physiology* 2001; 281: C1285-C1290.
35. Zhang H, Audira G, Li Y, Xian W, Varikkodan MM, Hsiao CD. Comparative study the expression of calcium cycling genes in Bombay duck (*Harpadon nehereus*) and beltfish (*Trichiurus lepturus*) with different swimming activities. *Genom Data* 2017;12:58-61.
36. Gergely J. Professor Ebashi's impact on the study of the regulation of striated muscle contraction. *Mol Cell Biochem* 1999; 190: 5-8.
37. Germinario E, Esposito A, Megighian A, Midrio M, Danieli-Betto D. Early changes of type 2B fibers after denervation of rat EDL skeletal muscle. *J Appl Physiol* 2002; 92: 2045-2052.
38. Leeuw T, Kapp M, Pette D. Role of innervation for development and maintenance of troponin subunit isoform patterns in fast- and slow-twitch muscles of the rabbit. *Differentiation* 1994; 55: 193-201.
39. Ruegg DG, Kakebeeke TH, Gabriel JP, Bennefeld M. Conduction velocity of nerve and muscle fiber action potentials after a space mission or a bed rest. *Clin Neurophysiol* 2003; 114: 86-93.
40. Tieland M, Trouwborst I, Clark B C. Skeletal muscle performance and ageing. *Journal Cachexia Sarcopenia Muscle* 2018; 9:3-19.
41. Ruegg DG, Kakebeeke TH, Gabriel JP, Bennefeld M. Effects of ageing on single muscle fibre contractile function following short-term immobilisation. *J Physiol* 2011; 589: 4745-4757.
42. Manzano R, Toivonen JM, Calvo AC, Muñoz MJ, Zaragoza P, Osta R. Housekeeping gene expression in myogenic cell cultures from neurodegeneration and denervation animal models. *Biochem Biophys Res Commun* 2011; 407: 0-763.
43. Nakao R, Yamamoto S, Yasumoto Y, Kadota K, Oishi K. Impact of denervation-induced muscle atrophy on housekeeping gene expression in mice. *Muscle Nerve* 2015; 51: 276-281.