

Original article

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Effect of a preparation of branched-chain amino acids on the regeneration of the anterior tibial muscle after compression injury

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Abstract

Introduction Histological data on the effect of intramuscular administration of branched-chain amino acids (BCAA) on muscle recovery are lacking.

The **purpose** of the work was a histomorphometric assessment of the effect of intramuscular administration of BCAA in a dosage for injections on the regeneration of the anterior tibial muscle (ATM) after its closed partial crushing in rats.

Materials and methods Compression of the ATM was performed for one minute in 48 male Wistar rats. After 24 hours, animals of series 1 received a single injection of saline into the ATM, while animals of series 2 received BCAA. Euthanasia was performed after 7, 14, 28, and 42 days. In paraffin section images, the volumetric densities of muscle fibers (MF) (V_{Vm}), microvessels (V_{Vmv}), and endomysium (V_{Ve}) were determined; MF diameters were measured, the numerical densities of MF (Na_m), microvessels (Na_{mv}), and intramuscular nuclei (Na_n) were determined, and the vascularization (kv as Na_{mv}/Na_m) and nucleation (kn as Na_n/Na_m) coefficients were calculated. Ten intact rats were a control group.

Results After seven days, V_{Vm} in both series decreased by 36–37 %; in the rest of the periods, the indicator was significantly higher in series 2, but remained below normal in both groups. Within 28 days, the MF diameter was higher in series 2. After seven, 14 and 28 days, the indicator exceeded the values of series 1 by 17 % ($p = 0.026$), 11 % ($p = 0.012$) and 5 % ($p = 0.048$); after 28 days in series 1 and 2 it was 79 % and 84 % of the norm. The kn indicator in series 2 was lower than normal only after seven days of experiment, and in series 1 after seven, 14 and 42 days by 24 % ($p = 0.002$), 24 % ($p = 0.004$) and 10 % ($p = 0.034$), that indicates less active myogenesis. The kv indicator after 14 and 28 days was higher in series 2 by 10 % ($p = 0.045$) and 32 % ($p = 0.014$). On the 42nd day in series 2, the MF diameter decreased against the increase in their Na_m and was 47 % lower than the norm ($p = 0.0006$) and 30 % lower than in series 1 ($p = 0.0023$).

Discussion The findings obtained suggest that a BCAA injection has a positive effect on the MF regeneration and microvessels, and was more active than in series 1.

Conclusion A single BCAA injection has a myogenic stimulating effect. Further studies on side effects and long-term outcomes are needed.

Keywords: rat, crush injury, lower leg, tibialis anterior, histomorphometry, branched-chain amino acids

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INTRODUCTION

Treatment of musculoskeletal injuries remains a complex medical problem [1–3]. Skeletal muscle injuries account for approximately 30 % [4], and modern treatment methods in most cases are aimed at relieving pain and inflammation using nonsteroidal anti-inflammatory drugs, immobilization, and external physical stimulation [5, 6]. Severe injuries result in rhabdomyolysis (destruction of skeletal muscles), characterized by damage to muscle cells with the release of myoglobin and sarcoplasmic proteins into the extracellular fluid [7, 8]. The most common causes of rhabdomyolysis include compression injuries [9], in which ischemic necrosis is observed in skeletal muscle tissue, followed by the development of granulation tissue and the formation of muscle-connective tissue regenerate [10]. Despite the ability of skeletal muscles to regenerate thanks to the stem cell population [11, 12], the available reserve of myosatellite cells in such severe injuries usually does not ensure complete histo- and organotypic regeneration [13, 14], which leads to the loss of contractile tissue, its replacement with adipose and fibrous scar tissue, causing a long-term deficit in muscle structure and strength [5]. Thus, proper regeneration of skeletal muscles plays a key role in the restoration of their function after injury [15].

The available treatment methods remain insufficiently effective [16], which determines the demand for fundamental research on the development of new methods for inducing reparative rhabdomyogenesis and reducing fibrosis in clinical practice [5, 13, 14]. Numerous recent studies have focused on the investigation of the molecular mechanisms underlying muscle regeneration [17], on the search for activators of myogenesis of muscle progenitor cells and angiogenesis of endothelial cells [18, 19]. It is known that essential branched chain amino acids (BCAA), leucine, isoleucine and valine, possess such properties. They are potent regulators of protein synthesis in healthy skeletal muscles and in muscle atrophy; and leucine is used as a "pharmaceutical nutrient" to prevent atrophy and restore muscle in the elderly [20]. As dietary supplements, BCAAs have found wide application in repairing muscle damage and alleviating exercise-induced delayed-onset muscle soreness [21]. Intraperitoneal administration of leucine to mice has shown to reduce muscle atrophy and promote muscle mass maintenance during sepsis [22]. Orthopedic surgery and associated events (immobilization or prolonged muscle inactivity) result in significant muscle loss and long-term functional decline [23, 24]. Oral BCAA supplementation in such patients has improved muscle quality and mass compared to placebo [25, 26]. There is some positive experience with perioperative intravenous amino acid administration to patients undergoing elective total hip arthroplasty to compensate for surgically induced skeletal muscle protein loss [27, 28]. Data on preclinical studies of the effectiveness of intramuscular administration of BCAA in the area of damage are rare [29], and histological data on its effect on muscle regeneration are absent in the relevant literature, which determined the purpose of this work.

The **purpose** of the work was a histomorphometric assessment of the effect of intramuscular administration of BCAA in a dosage for injections on the regeneration of the anterior tibial muscle (ATM) after its closed partial crushing in rats.

MATERIALS AND METHODS

Study design

The study is interventional (experimental), single-center, prospective, selective, controlled, randomized.

Description of the experiment

Closed partial crushing of the anterior tibial muscle (ATM) using surgical forceps was performed on 48 Wistar rats (male, eight months old, weighing 462 (454; 477) g). The animals were divided into two groups: animals in series 1 ($n = 24$) received a single injection of saline into the ATM, while animals in series 2 ($n = 24$) were injected a preparation of branched chain amino acids.

To standardize the experimental model, the forceps were positioned perpendicular to the longitudinal axis of the tibia so that their jaws, when closed, exerted pressure on the tissue in the middle ATM belly. The forceps rings were directed cranially, and the clamp-lock was snapped into place in the same position for one minute in all cases. The experimental model is fully reproducible [30].

The procedure was performed in the operating room under general anesthesia using Rometar 2 % (Bioveta, Czech Republic) and Zoletil 100 (Virbac Sante Animale, France). After 24 hours, the animals in series 1 were injected with 0.02 ml of saline into the injured muscle area, while animals in series 2 were injected with 0.02 ml of solution of amino acids in saline (L-valine: L-isoleucine: L-leucine in equal weight ratios, 0.04 g of each amino acid in 10 ml of saline). In both series, injections were single.

The rats were then kept in cages, two in each cage, and were withdrawn from the experiment 7, 14, 28 and 42 days after the injury ($n = 6$ in each series at all time-points of the experiment).

Methods: ATM fragments, excised from the area of compression injury, were fixed in 10 % neutral formalin and embedded in paraffin. Transverse paraffin sections (5 μm thick) were prepared on a Thermo Scientific HM 450 microtome (USA) and stained with Masson's three-color method, hematoxylin, and eosin. After aldehyde-osmium fixation, a portion of the material was embedded in Araldite. Semithin sections (1 μm) were prepared on a Nova LKB ultramicrotome (Sweden) and stained with methylene blue, azure II, and basic fuchsin.

Full-color digital images were obtained using an AxioScope.A1 microscope and an AxioCam digital camera (Carl Zeiss MicroImaging GmbH, Germany). Using the electronic version of the original equidistant point test grid [31] in PhotoFiltre 7.0 software, stereological parameters were calculated on the 14th, 28th, and 42nd days after injury: volumetric densities of muscle fibers (V_{vm}), microvessels (V_{vmv}) and endomesium (V_{ve}). The VideoTesT Master-Morphology 4.0 software (Russia) was used to measure the average diameter of muscle fibers (up to 400 for each animal), determined the numerical density of muscle fibers (Na_m), microvessels (V_{vmv}), and intramuscular nuclei (Na_m) per 1 mm^2 of area, and calculated the vascularization coefficients (kv as Na_{mv} / Na_m) and nucleation coefficients (kn as Na_n / Na_m). The proportion (%) of reactive-destructive altered myelinated nerve fibers in the total sample volume of intramuscular nerve trunks was determined. Ten intact male rats, 8–9 months old, were a control group.

During the experiment, the rats' motor activity was assessed in the open field test. At one and the same time (11:00 AM), the animals were placed in a circular arena 120 cm in diameter, the floor of which was marked with 12-cm squares. After the animals adapted (one minute after placement), video recording was performed for one minute, recording the total number of square crossings.

Ethical statement

The study was approved by the institutional ethics committee (protocol dated November 29, 2024 No. 1(76)), conducted in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes and Directive 2010/63/EU of the European Parliament and the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes.

Statistical analysis

The sample size was calculated based on the minimum number necessary to obtain reliable results. Statistical data processing was performed using the AtteStat version 9.3.1 software program (developed by I.P. Gaidyshev, Rospatent registration certificate No. 2002611109, Russia). The Kolmogorov test was used to check the normality of the sample distribution. The Mann – Whitney test was used for pairwise comparisons of animal groups. Differences were considered significant at $p < 0.05$.

RESULTS

The motor activity indices according to the open field test in the rats of series 1 by the end of the observation period (day 42) were significantly lower than the initial values and amounted to 13.0 (12.0–14.0) square intersections versus the initial 19.5 (16.8–24.3), $p = 0.007$. In series 2, on the 28th day after the injury, statistically significant differences with the initial indices were absent: 15.5 (12.3–18.0) square intersections versus the initial 18.5 (15.8–26.0), $p > 0.05$.

Seven days after the injury, a similar morphological picture was revealed in the ATM of both series: destructively altered muscle fibers (MF), regenerating MF and areas of granulation tissue were found in the surface layer and the area of adjacent muscle to the tibia. In cross sections, disorganization of part of the fascicles, swelling of the endomysium, its hypervascularization, and increased cellularity with a predominance of fibroblasts, fibrocytes and macrophages are visible. MFs had a smaller diameter and rounded contours (Fig. 1 a, b). Intramuscular nuclei were located both on the periphery, solely, sometimes in chains or conglomerates, and in the central part of the fibers; some of the regenerating fibers were at the myotube stage (Fig. 1 c). After seven days of the experiment, myelinated nerve fibers of intramuscular nerve trunks changed destructively in both series (Fig. 1 e). In longitudinal sections at this stage of the experiment, MFs without transverse striations were found, there were areas with wavy, crimped MFs of uneven thickness, some of them with impaired integrity and areas of necrobiosis, thin regenerating MFs with chains or agglomerated nuclei, fibers in the myotube stage (Fig. 1 d) and rare MFs expanded at the ends with clusters intramuscular nuclei ("muscle buds") (Fig. 1 f).

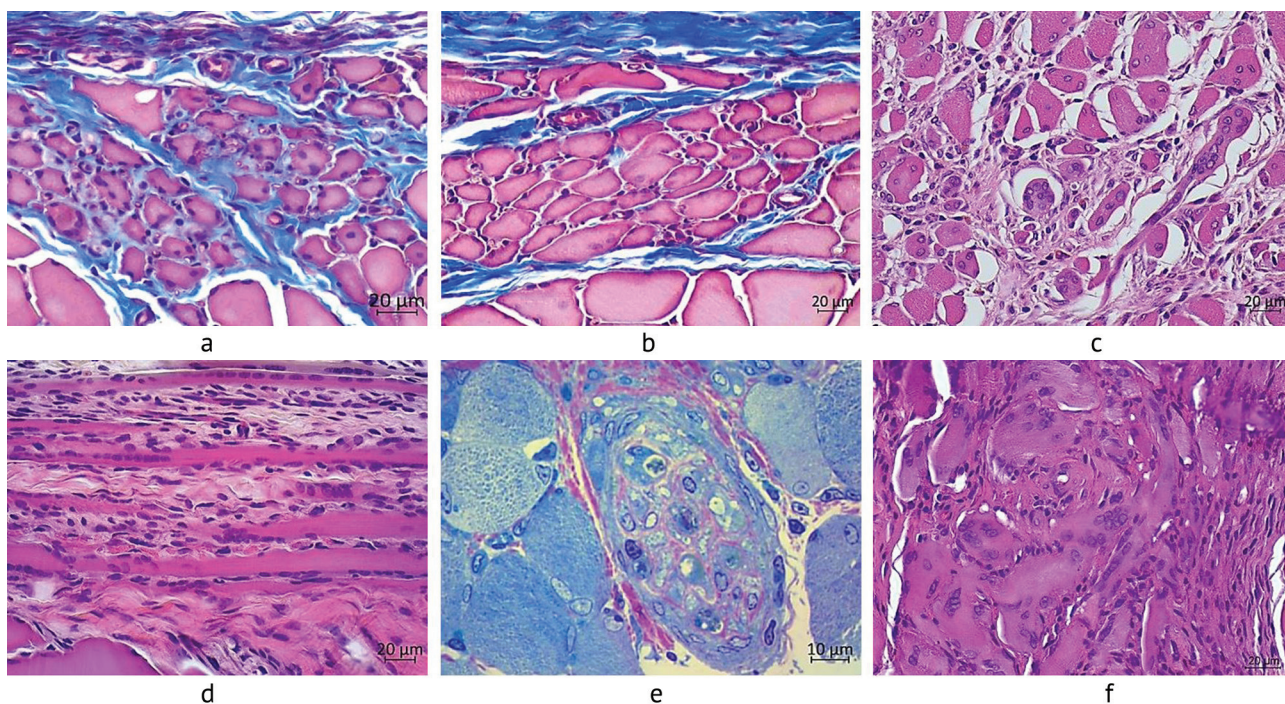


Fig. 1 Anterior tibial muscles of rats after seven days (a – series 1; b–e – series 2) and 14 days (f – series 2) after compression injury. Transverse paraffin sections, stained with hematoxylin and eosin (c, d, f) and Masson's three-color method (a, b), magnification 400 $\times$. Transverse semithin section (e), stained with methylene blue, azure II, and basic fuchsin, magnification 1000 $\times$

After 14 and 21 days of the experiment, regenerative processes predominated in the superficial and deep zones of both series. The MFs were predominantly rounded in shape with peripheral or central nuclei (Fig. 2 a, b); myotubes continued to be present, and in longitudinal sections, corrugated fibers were observed, club-shaped at the ends of the MFs with clusters of nuclei ("muscle buds") (Fig. 1 e). The interstitial spaces remained dilated with an increased content of fibroblasts and fibrocytes.

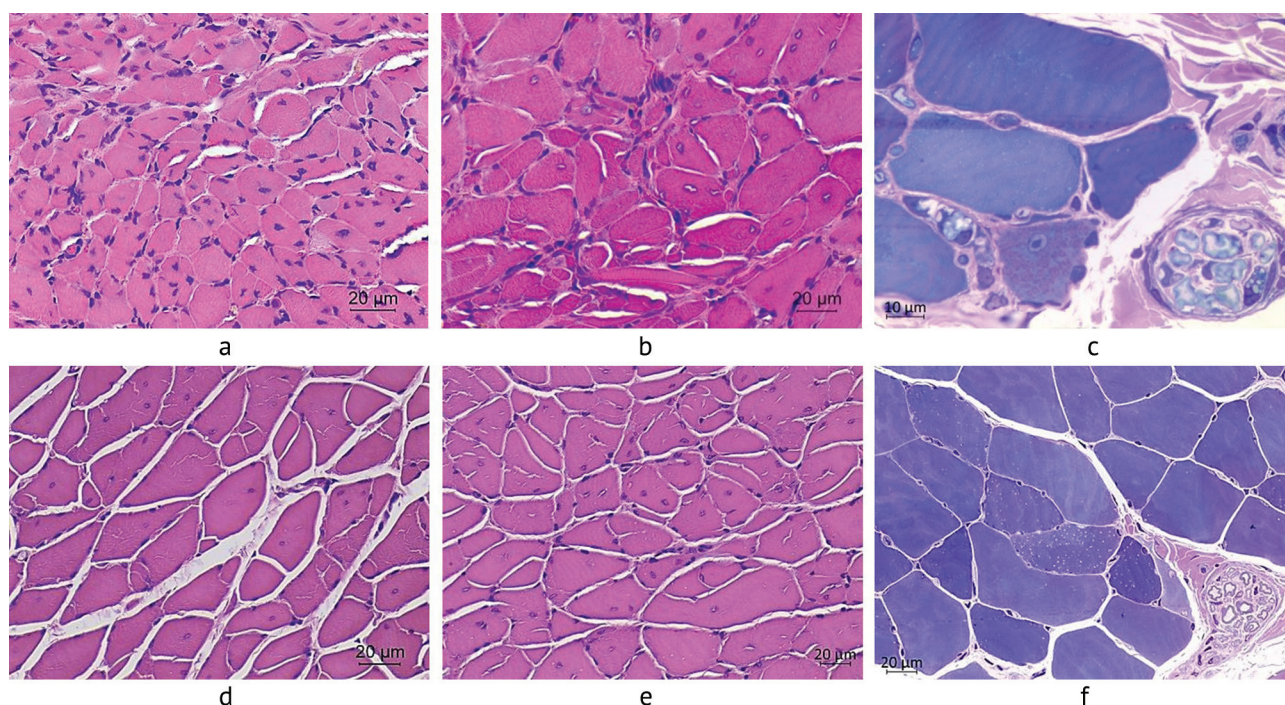


Fig. 2 Anterior tibial muscles of rats after 21 days (a — series 1; b, c — series 2) and on the 42nd day (d — series 1; e, f — series 2) after compression injury. Transverse paraffin sections, stained with hematoxylin and eosin (a, b, d, e), magnification 400×. Transverse semi-thin sections (c, f), stained with methylene blue, azure II and basic fuchsin, magnification 1000× (c), 400× (f)

On the 42nd day after injury, the MFs were polymorphic in the superficial and deep zones; there were small regenerating fibers with rounded contours, and some of the MFs acquired a polygonal shape. The nuclei occupied both peripheral and central locations, interstitial spaces remained expanded (Fig. 2 d, e) in both series.

In the intramuscular nerve trunks, after 14 days, destructively altered nerve fibers predominated, and after 21 days and at the end of the experiment, nerve fibers of normal structure predominated (Fig. 2 c, f).

In the layer of the anterior tibial muscle between the superficial and deep zones, the fascicular structure was preserved in both series at all stages of the experiment, but the endomysial layers were thickened. Some of the MFs had a normal structure and a polygonal shape, while others had rounded or deflated contours shifted to the center of the nucleus, uneven staining, and vacuolization. Dystrophic changes became less pronounced toward the end of the experiment.

Histomorphometric study of the size and numerical characteristics of the MFs showed that after seven days in series 1 and 2 their median diameter was reduced (Fig. 3 a) by 50 % ($p = 0.0001$) and 39 % ($p = 0.003$), and their numerical densities exceeded the norm by 2.8 ($p = 0.00002$) and 1.7 ($p = 0.0005$) times, respectively (Fig. 3 b). After 14 days of the experiment, the MF diameter in series 1 and 2 increased relative to the previous period and amounted to 59 % ($p = 0.001$) and 67 % ($p = 0.0013$) of the norm, respectively (Fig. 3 a). The numerical density of MFs in series 2 increased, and in both series it exceeded the norm ($p = 0.0002$) by 2.6 times (Fig. 3 b).

A stereological study showed that in series 1 and 2, V_{vm} was reduced ($p < 0.001$) relative to the parameters of the intact muscle by 37.6 % and 36.5 %; V_{ve} was increased by 34.7 % and 34.2 %; V_{mv} was increased by 2.5 and 2.2 times, respectively (Table 1). Comparison of series 1 and 2 did not reveal significant differences in these parameters.

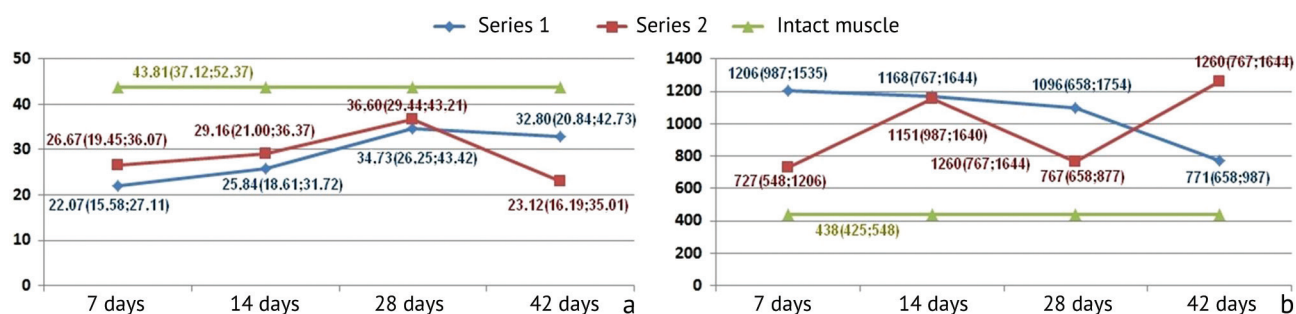


Fig. 3 Diagram of changes in the diameter (μm) of muscle fibers (a) and the numerical density (in 1 mm² of section area) of muscle fibers (b) of the anterior tibial muscles of series 1 and 2 at the stages of the experiment (Me (Q1; Q3))

After 28 days, the diameter of the MFs in series 1 and 2 increased and was 79 % ($p = 0.007$) and 84 % ($p = 0.004$) of the norm (Fig. 3 a), and the numerical density of MFs decreased relative to the previous period, but exceeded the norm by 2.5 ($p < 0.001$) and 1.8 ($p = 0.006$) times, respectively (Fig. 3 b).

Stereological analysis showed that V_{vm} increased in series 1 and 2 by 6.8 % and 11.3 %, but remained low ($p < 0.001$), and V_{ve} and V_{vm} remained elevated ($p < 0.001$) relative to intact values (Table 1). A comparison of the experimental series showed that in series 2 V_{vm} was 5.6 % higher, and V_{ve} was 5.5 % lower ($p < 0.001$) than the values of series 1 (Table 1), but there were no significant differences in V_{vmv} (Table 1).

At the end of the experiment (42nd day), the diameter of the MFs in series 1 was not restored and was 75 % ($p = 0.0005$) of the norm (Fig. 3 a), and the numerical density of Mfs was increased ($p = 0.006$) by 1.8 times (Fig. 3 b). In series 2, the numerical density of MFs increased, and the diameter decreased relative to the previous period, amounting to 53 % ($p = 0.0006$) of the norm (Fig. 3 a, b). The ratio of the proportions of tissue components in both experimental series did not restore to normal values: V_{vm} remained reduced ($p < 0.001$), but in series 2, as after 28 days, the value of V_{vm} was higher than in series 1 by 5.7 % ($p < 0.001$), and V_{ve} was lower ($p < 0.001$) by 5.6 % (Table 1). There were no significant differences in the V_{vmv} parameter between series 1 and 2.

Table 1

Volumetric densities of tissue components (%) of the intact anterior tibial muscle in experimental series ones at the stages of the experiment ($M \pm \sigma$)

Term	Series	Volumetric density		
		Muscle fibers	Microvessels	Endomysium
14 days	Series 1	54.9 ± 3.2 ; $p^n = 4 \cdot 10^{-8*}$; $p^{1-2} = 0.184$	5.0 ± 1.4 ; $p^n = 9 \cdot 10^{-7*}$; $p^{1-2} = 0.069$	40.2 ± 3.4 ; $p^n = 4 \cdot 10^{-8*}$; $p^{1-2} = 0.273$
	Series 2	56.0 ± 13.1 ; $p^n = 4 \cdot 10^{-8*}$	4.4 ± 0.8 ; $p^n = 1 \cdot 10^{-7*}$	39.7 ± 12.9 ; $p^n = 4 \cdot 10^{-8*}$
28 days	Series 1	61.7 ± 6.4 ; $p^n = 4 \cdot 10^{-8*}$; $p^{1-2} = 0.001^*$	4.8 ± 1.3 ; $p^n = 4 \cdot 10^{-7*}$; $p^{1-2} = 0.882$	33.5 ± 6.7 ; $p^n = 4 \cdot 10^{-8*}$; $p^{1-2} = 0.002^*$
	Series 2	67.3 ± 5.3 ; $p^n = 4 \cdot 10^{-8*}$	4.8 ± 0.9 ; $p^n = 9 \cdot 10^{-8*}$	28.0 ± 5.4 ; $p^n = 4 \cdot 10^{-8*}$
42 days	Series 1	60.4 ± 5.0 ; $p^n = 4 \cdot 10^{-8*}$; $p^{1-2} = 0.002^*$	4.4 ± 0.7 ; $p^n = 8 \cdot 10^{-8*}$; $p^{1-2} = 0.694$	35.3 ± 4.8 ; $p^n = 4 \cdot 10^{-8*}$; $p^{1-2} = 0.001^*$
	Series 2	66.1 ± 4.6 ; $p^n = 4 \cdot 10^{-8*}$	4.3 ± 0.7 ; $p^n = 4 \cdot 10^{-8*}$	29.7 ± 4.6 ; $p^n = 4 \cdot 10^{-8*}$
Intact muscle		92.5 ± 1.6	2.0 ± 0.7	5.5 ± 1.4

* — differences between the experimental series and the norm (p^n), series 1 and 2 (p^{1-2}) were considered statistically significant according to the Mann – Whitney test at $p < 0.05$

The analysis of the distribution of MFs by diameters showed that in both series at all stages of the experiment the histograms retained a unimodal character, but their bases shifted to the left relative to the norm (Fig. 4). After seven days in series 1 (Fig. 4 a), the proportion of small regenerating and atrophied muscle fibers $D < 30 \mu m$ was 84 % (normally — single, 1 %), by the 42nd day it decreased to 44 %, and large fibers $D > 50$ at all stages (Fig. 4) account for 1–2 % (normally — 62 %). In Series 2,

after seven days (Fig. 4 a), the proportion of small fibers $D < 30 \mu\text{m}$ is 60 %, decreases after 28 days to 29 %, but increases again by the 42nd day of the experiment to 67 %, and large muscle fibers $D > 50$ at all stages account for 3-5 % (Fig. 4). At the end of the experiment in series 2, the mode shifts to the left (Fig. 4 d) into the range of smaller values of 11–20 μm (series 1, 31–40 μm).

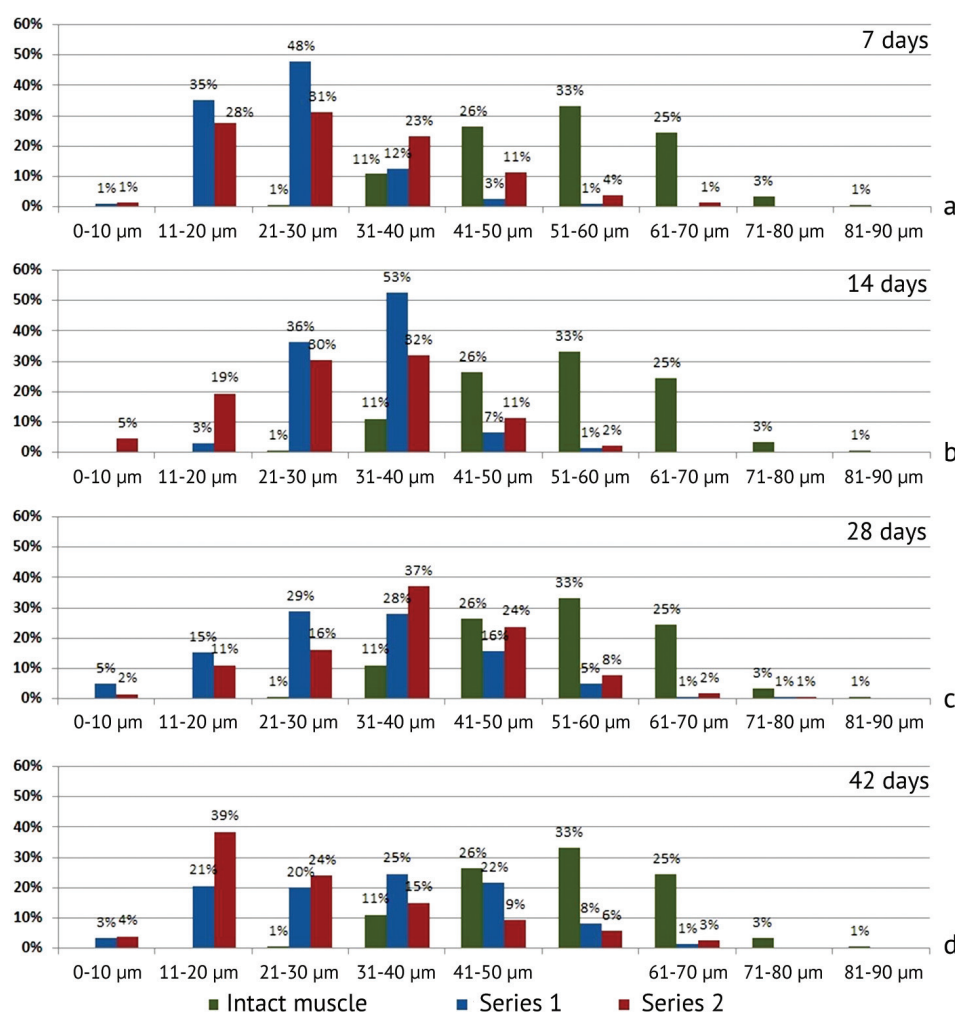


Fig. 4 Histograms of the distribution of muscle fibers of the anterior tibial muscle by diameter in rats of the intact group, series 1 and series 2 after seven (a), 14 (b), 28 (c) and 42 (d) days of the experiment. The X-axis is the fiber diameters (μm), the Y-axis is the percentage of fibers in the sample

The Na_{mv} and Na_n parameters of 1 mm^2 exceed the norm in both series at all periods of the experiment (Table 2), but this kv in Series 1 remains reduced after seven, 14, and 28 days by 1.6, 1.6 and 1.3 times, respectively, and exceeds the norm by 1.3 times only on the 42nd day (Table 2). In series 2, after 7 and 14 days kv was reduced by 1.6 and 1.4 times, after 28 it exceeded the norm by 1.1 times, but on day 42 it was reduced by 1.9 times (Table 2). It should be noted that after seven days there were no significant differences in this parameter between the series; after 14 and 28 days, the vascularization coefficient was significantly increased in series 2, but by the end of the experiment its value becomes 2.3 times lower than in series 1 (Table 2).

The nucleation coefficient in Series 1 was reduced by 1.3 times within 14 days, increased by 28 days and exceeded the norm by 1.2 times, and decreased by 1.1 times on the 42nd day (Table 2). In Series 2, this indicator was reduced by 1.2 times only after seven days of the experiment, after 14 days it exceeded the norm by 1.1 times, and at the remaining time points it had normal values (Table 2). The nucleation coefficient in Series 2 exceeded the values of Series 1 after 14 days by 1.4 times, and became lower by 1.3 times on the 28th day; at the end of the experiment, there were no reliable differences between the series (Table 2).

Table 2

Quantitative indices of vascularization and nucleation of the intact anterior tibial muscle and the experimental one at the stages of the experiment: Me (Q1; Q3)

Term	Series	Na_{mv}	Na_n	kv	kn
7 days	Series 1	1315 (981; 1526) $p^n = 0.0001^*$ $p = 0.02^*$	1370 (877; 1755) $p^n = 0.0015^*$ $p = 0.002^*$	1.11 (0.89; 1.19) $p^n = 0.0031^*$ $p = 0.09$	1.18 (0.87; 1.38) $p^n = 0.002^*$ $p = 0.049$
7 days	Series 2	1041 (658; 1315) $p^n = 0.002^*$	877 (658; 1863) $p^n = 0.004^*$	1.12 (0.91; 1.73) $p^n = 0.003^*$	1.29 (1.00; 1.62) $p^n = 0.0035^*$
14 days	Series 1	1261 (987; 1535) $p^n = 0.0002^*$ $p = 0.04^*$	1370 (1206; 1754) $p^n = 0.00002^*$ $p = 0.032^*$	1.08 (0.89; 1.29) $p^n = 0.002^*$ $p = 0.045^*$	1.18 (1.00; 1.33) $p^n = 0.004^*$ $p = 0.033^*$
14 days	Series 2	1480 (1315; 1644) $p^n = 0.00002^*$	1863 (1644; 2083) $p^n = 0.00033^*$	1.20 (0.83; 1.70) $p^n = 0.0026^*$	1.63 (1.31; 1.88) $p^n = 0.052$
28 days	Series 1	1425 (987; 2192) $p^n = 0.0013^*$ $p = 0.056$	1809 (1315; 2631) $p^n = 0.00005^*$ $p = 0.029^*$	1.31 (1.00; 1.60) $p^n = 0.020^*$ $p = 0.014^*$	1.88 (1.36; 2.20) $p^n = 0.036^*$ $p = 0.03^*$
28 days	Series 2	1535 (1096; 1644) $p^n = 0.0002^*$	1096 (877; 1315) $p^n = 0.003^*$	1.93 (1.25; 2.14) $p^n = 0.03^*$	1.50 (1.29; 1.55) $p^n = 0.131$
42 days	Series 1	1754 (1206; 1836) $p^n = 0.008^*$ $p = 0.028^*$	1096 (767; 1206) $p^n = 0.010^*$ $p = 0.003^*$	2.20 (1.43; 2.60) $p^n = 0.01^*$ $p = 0.006^*$	1.41 (1.00; 1.70) $p^n = 0.034^*$ $p = 0.044^*$
42 days	Series 2	987 (767; 1535) $p^n = 0.04^*$	1762 (1425; 2521) $p^n = 0.005^*$	0.94 (0.64; 1.31) $p^n = 0.008^*$	1.53 (0.94; 2.60) $p^n = 0.371$
Intact group		767 (658; 1096)	655 (532; 877)	1.75 (1.33; 2.33)	1.56 (1.20; 2.00)

Note: Na_{mv} is the numerical density of muscle fibers; Na_n is the numerical density of microvessels; Na_n is the numerical density of intramuscular nuclei (Nan) in 1 mm² of cross-sectional area; kv is the vascularization coefficient (Na_{mv} / Na_n), kn is the nucleation coefficient (Na_{mv} / Na_n). * — differences between the experimental series and the norm (p^n), series 1 and 2 (p) were considered statistically significant according to the Mann – Whitney test at $p < 0.05$.

Morphometric study of intramuscular nerve trunks showed that after seven days of the experiment the proportion (%) of destructively altered myelinated nerve fibers in series 1 and 2 was $(91.8 \pm 4.5) \%$ and $(94.4 \pm 6.1) \%$, after 14 days — $(88.2 \pm 5.3) \%$ and $(80.0 \pm 7.2) \%$, after 21 days — $(27.3 \pm 2.8) \%$ and $(33.4 \pm 6.4) \%$, and at the end of the experiment (42nd day) — $(8.9 \pm 3.1) \%$ and $(9.5 \pm 2.2) \%$, respectively.

DISCUSSION

Our study assessed the effects of a single intramuscular injection of a BCAA preparation developed by the authors in an injectable dosage into the closed partial crush ATM zone in rats on regenerative processes. Previously, a single administration at the studied concentration demonstrated its safety in rats, and lower serum creatine phosphokinase activity (a marker of muscle damage) was observed in animals injected with this preparation compared to controls [29].

It is known that the compression injury of skeletal muscles due to mechanical pressure and ischemia-reperfusion injures not only muscle fibers but also blood vessels and nerves [30, 32], extensive degradation of which is observed during the degenerative and inflammatory phases [33, 34]. Activation of myogenesis of muscle progenitor cells and angiogenesis of endothelial cells is crucial for muscle regeneration after injury [35]. There is evidence that essential branched chain amino acids (leucine, isoleucine and valine (BCAA)) are activators of reparative rhabdomyogenesis and angiogenesis [20].

Our stereological study of changes in the ratio of ATM tissue components in rats regenerating after compression injury revealed an equivalent decrease in the proportion of muscle fibers (by 36–37 %) and an increase in the proportion of endomysium in both series after seven days. At other time-points, the volume fraction of muscle fibers was significantly higher in the series with BCAA injection, but at the end of the experiment remained below normal in both groups. The obtained data are consistent with the results of stereological studies by Shchudlo et al. [30], who, using

the same experimental model of closed partial crushing of the rat ATM, but with a shorter duration of compression (20 s), did not reveal a complete restoration of the volume fraction of muscle fibers even 90 days after the injury.

To assess the effectiveness of muscle regeneration after traumatic and denervation injuries, the indicator "average or median diameter of muscle fibers" is most often used [30, 36–38]. Our quantitative analysis of the population of muscle fibers regenerating after closed partial crushing showed that during 28 experimental days their median diameter was significantly higher in the series with the BCAA injection. Moreover, in that series, the nucleation index (the number of nuclei in the cross-sectional profile of one muscle fiber) was significantly reduced relative to the norm only after seven days of the experiment, and in the series without the injection after seven, 14 and 42 days. Lower values of this indicator in the series with natural regeneration may indicate less active myogenesis, since, according to the literature, it is the activation of myosatellite cells that is one of the main factors determining the ability of skeletal muscle to regenerate [39]. At the end of the experiment (day 42), the diameter of muscle fibers in both series did not reach normal values and even decreased, but was significant only in the series with BCAA injection, in which the proportion of small fibers with a diameter of 10–30 μm increases simultaneously with an increase in the numerical density and higher values of the volumetric density of muscle fibers than in series 1. Such dynamics may be associated with persistent atrophy of some muscle fibers, a hyperplastic regenerative response in the form of myon splitting and their dystrophic changes [30], associated with impaired blood supply and innervation. Thus, in our study, within 28 days, the muscle fiber vascularization index (the number of vessels supplying one muscle fiber) was significantly higher in the series with BCAA, but by the 42nd day after the injury it was lower than the values of series 1 and the norm. It is known that the rate of muscle regeneration and the completeness of muscle tissue restoration directly depend on angiogenesis and restoration of the microvascular network [40]. Dense vasculature may be more important for rapid repair of muscle damage than abundance of satellite cells, and lower capillary density may impair regeneration [41].

Our study showed that the innervation of the skeletal ATM in the projection of the compression groove was impaired in both series to the same extent due to the destruction of the myelinated nerve fibers of the intramuscular nerve trunks and was not restored by the end of the experiment, which is also one of the causes of muscle fiber atrophy [42], which persisted until the end of the experiment. Denervated skeletal muscles lose their contractile function, which leads to decreased blood perfusion and a state of muscle hypoxia, excessive production of reactive oxygen species, and muscle fiber damage due to oxidative stress [43].

Thus, the results of our morphological, stereological, and morphometric studies of the ATM after compression injury suggest that a single BCAA injection has a positive effect on muscle fiber and microvascular regeneration. It is more effective than spontaneous regeneration.

Further research is needed on the effect of injectable BCAA on muscle regeneration, with adjustments to dosage, schedule, number of injections, study of adaptive responses, potential side effects, and long-term results to confirm its effectiveness.

CONCLUSION

A single BCAA intramuscular injection of the original preparation based on branched chain amino acids (BCAA) in the medical injection dosage into the area of a partial crush injury of the anterior tibial muscle has a myogenic stimulating effect.

Conflict of interest The authors declare that they have no competing interests related to the study and its publication.

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REFERENCES

1. Belokrylov NM, Ladeischikov VM, Schekolova NB, et al. Damages of locomotor apparatus in children as a result of road accidents. *Perm Medical Journal*. 2018;35(4):26–38. (In Russ.) doi: 10.17816/pmj35426–38.
2. Kovrizhnykh YA, Kovrizhnykh MV, Zapariy NS. Main Trends in Recurrent Disability Resulting from Musculoskeletal Injury Outcomes in Moscow in 2012–2017. *Public Health and Life Environment – PH&LE*. 2021;(1):28–33. (In Russ.) doi: 10.35627/2219–5238/2021–334–1–28–33.
3. Zorin VI, Lukyanov SA. Telemedicine consultations for children with musculoskeletal injuries: data from the Turner National Medical Research Center of children's orthopedics and trauma surgery. *Traumatology and Orthopedics of Russia*. 2023;29(3):86–93. doi: 10.17816/2311–2905–11175.
4. Nakata K., Ishikawa M., Kamei N, et al. Skeletal muscle injury treatment using the Silk Elastin® injection in a rat model. *Regen Ther*. 2024;(26):180–187. doi: 10.1016/j.reth.2024.05.012.
5. Marmolejo-Martínez-Artesero S, Romeo-Guitart D, Venegas V, et al. NeuroHeal Improves Muscle Regeneration after Injury. *Cells*. 2020;10(1):22. doi: 10.3390/cells10010022.
6. Amaechi O, Huffman MM, Featherstone K. Pharmacologic Therapy for Acute Pain. *Am Fam Physician*. 2021;104(1):63–72.
7. Grover KM, Sripathi N. Rhabdomyolysis. *Muscle Nerve*. 2026;73(4):527–533. doi: 10.1002/mus.70079.
8. Yang BF, Li D, Liu CL, et al. Advances in rhabdomyolysis: A review of pathogenesis, diagnosis, and treatment. *Chin J Traumatol*. 2026;29(1):21–31. doi: 10.1016/j.cjtee.2024.10.005.
9. Mokhtari AK, Maurer LR, Christensen MA, et al. Rhabdomyolysis in Severe COVID-19: Male Sex, High Body Mass Index, and Prone Positioning Confer High Risk. *J Surg Res*. 2021;266:35–43. doi: 10.1016/j.jss.2021.03.049.
10. Shperling IA, Odintsova IA, Shulepov AV, et al. Features of regenerative histogenesis of skeletal muscles in the area of compression in experimental compression trauma. *Clinical pathophysiology*. 2021;27(S3):27. (In Russ.)
11. Scala P, Rehak L, Giudice V, et al. Stem Cell and Macrophage Roles in Skeletal Muscle Regenerative Medicine. *Int J Mol Sci*. 2021;22(19):10867. doi: 10.3390/ijms221910867.
12. Wang DA, Li QZ, Jia DM. Low-Frequency Electrical Stimulation Promotes Satellite Cell Activities to Facilitate Muscle Regeneration at an Early Phase in a Rat Model of Muscle Strain. *Biomed Res Int*. 2021;2021:4218086. doi: 10.1155/2021/4218086.
13. Chernova ON, Korsakov IN, Samchuk DP, et al. Experimental models for studying of skeletal muscles regeneration. *Genes & Cells*. 2015;10(4):127–140. (In Russ.) doi: 10.23868/gc120536.
14. Deev RV, Bozo IY, Mavlikeev MO, et al. Regenerative histogenesis in a skeletal muscle defect with local implantation of gene-activated hydrogel based on hyaluronic acid in the experiment. *Genes & Cells*. 2020;15(2):66–72. (In Russ.) doi: 10.23868/202004015.
15. Wu Z, Xu H, Xu Y, et al. Andrographolide promotes skeletal muscle regeneration after acute injury through epigenetic modulation. *Eur J Pharmacol*. 2020;888:173470. doi: 10.1016/j.ejphar.2020.173470.
16. Contreras-Muñoz P, Torrella JR, Venegas V, et al. Muscle Precursor Cells Enhance Functional Muscle Recovery and Show Synergistic Effects With Postinjury Treadmill Exercise in a Muscle Injury Model in Rats. *Am J Sports Med*. 2021;49(4):1073–1085. doi: 10.1177/0363546521989235.
17. Jin JB, Robinson A, Soukup T, et al. Metabolic and molecular regulation in skeletal muscle dysfunction and regeneration. *Front Cell Dev Biol*. 2025;13:1651553. doi: 10.3389/fcell.2025.1651553.
18. Koike H, Sugimura M, Ouchi R, et al. Macrophage Subpopulation Promotes Skeletal Muscle Regeneration Through HGF/MET Signaling-Mediated Skeletal Muscle Stem Cell Proliferation. *Aging Cell*. 2025;24(6):e70042. doi: 10.1111/accel.70042.
19. Hirano K, Nakabayashi C, Sasaki M, et al. Mg²⁺ influx mediated by TRPM7 triggers the initiation of muscle stem cell activation. *Sci Adv*. 2025;11(14):eadu0601. doi: 10.1126/sciadv.adu0601.
20. Wilkinson K, Koscienc CP, Monteyne AJ, et al. Association of postprandial postexercise muscle protein synthesis rates with dietary leucine: A systematic review. *Physiol Rep*. 2023;11(15):e15775. doi: 10.14814/phy2.15775.
21. Weber MG, Dias SS, de Angelis TR, et al. The use of BCAA to decrease delayed-onset muscle soreness after a single bout of exercise: a systematic review and meta-analysis. *Amino Acids*. 2021;53(11):1663–1678. doi: 10.1007/s00726-021-03089-2.
22. Hou YC, Wu JM, Chen KY, et al. Glutamine and leucine administration attenuates muscle atrophy in sepsis. *Life Sci*. 2023;314:121327. doi: 10.1016/j.lfs.2022.121327.
23. Hemstock R, Mulhall D, Didyk J, et al. Postoperative weight-bearing restrictions and rehabilitation protocols after hip arthroscopy for femoroacetabular impingement: a systematic review. *J Hip Preserv Surg*. 2023;10(3–4):220–227. doi: 10.1093/jhps/hnad023.
24. Cordingley DM, Taheri M, Fasihiyan M, et al. Selected Nutrients to Oppose Muscle Disuse Following Arthroscopic Orthopedic Surgery: A Narrative Review. *Nutrients*. 2025;17(7):1273. doi: 10.3390/nu17071273.
25. Ikeda T, Suzuki S, Aimoto K, et al. Effect and feasibility of the combination of branched chain amino acid and exercise therapy on muscle mass and echo intensity of muscle in orthopedic patients in a convalescent rehabilitation hospital: A crossover trial. *Health Sci Rep*. 2023;6(6):e1316. doi: 10.1002/hsr2.1316.
26. Witard OC, Hughes AK, Morgan PT, et al. Protein-based perioperative nutrition interventions for improving muscle mass and functional outcomes following orthopaedic surgery. *Exp Physiol*. 2025;110(12):1802–1809. doi: 10.1113/EP092237.
27. Church DD, Schutzler SE, Wolfe RR, Ferrando AA. Perioperative amino acid infusion reestablishes muscle net balance during total hip arthroplasty. *Physiol Rep*. 2021;9(18):e15055. doi: 10.14814/phy2.15055.
28. Khani Y, Salmani A, Elahi M, et al. Peri-operative protein or amino acid supplementation for total joint arthroplasty: a systematic review and meta-analysis. *J Orthop Surg Res*. 2025;20(1):439. doi: 10.1186/s13018-025-05847-4.
29. Stogov MV, Kononovich NA, Tushina NV, et al. Efficiency of a preparation of amino acids with branched-chain side radicals in the dosage form for injections in traumatic injury of skeletal muscles in the experiment. *Experimental and clinical pharmacology*. 2025;88(2):34–38. (In Russ.) doi: 10.30906/0869-2092-2025-88-2-34-38.

30. Shchudlo NA, Shchudlo MM, Kononovich NA. Histomorphometric characteristic of skeletal muscle regenerating after a closed partial crush injury. *Morfologiya*. 2014;146(4):59-63. (In Russ.)
31. Shchudlo MM, Stupina TA, Shchudlo NA. Quantitative analysis of articular cartilage metachromasia in telepathology. *News of the Chelyabinsk Scientific Center*. 2004;25(Special issue):17-22. (In Russ.)
32. Tu H, Zhang D, Corrick RM, et al. Morphological Regeneration and Functional Recovery of Neuromuscular Junctions after Tourniquet-Induced Injuries in Mouse Hindlimb. *Front Physiol*. 2017;8:207. doi: 10.3389/fphys.2017.00207.
33. Forcina L, Cosentino M, Musarò A. Mechanisms Regulating Muscle Regeneration: Insights into the Interrelated and Time-Dependent Phases of Tissue Healing. *Cells*. 2020;9(5):1297. doi: 10.3390/cells9051297.
34. Morton AB, Jacobsen NL, Segal SS. Functionalizing biomaterials to promote neurovascular regeneration following skeletal muscle injury. *Am J Physiol Cell Physiol*. 2021;320(6):C1099-C1111. doi: 10.1152/ajpcell.00501.2020.
35. Roux-Biejat P, Coazzoli M, Marrazzo P, et al. Acid Sphingomyelinase Controls Early Phases of Skeletal Muscle Regeneration by Shaping the Macrophage Phenotype. *Cells*. 2021;10(11):3028. doi: 10.3390/cells10113028.
36. Filimonova GN, Kubrak NV, Krasnov VV, Ryabykh SO. Histomorphometric study of the soleus muscle under conditions of modeling of spinal cord contusion injury: experimental morphological study. *Russian Journal of Spine Surgery*. 2021;18(4):111-118. (In Russ.) doi: 10.14531/ss2021.4.111-118.
37. Shchudlo NA, Kobzyev AE, Varsegova TN, Stupina TA. Histomorphometric assessment of the tibial nerve and small muscles of the foot after internal neurolysis and autogenous plastic surgery of the tibial portion of the sciatic nerve in rats. *Genij Ortopedii*. 2022;28(6):823-829. doi: 10.18019/1028-4427-2022-28-6-823-829.
38. Mokhovikov DS, Stupina TA, Varsegova TN et al. Histomorphometric characteristics of the tibialis anterior muscle and the peroneal nerve in experimental repair of post-resection tibial defect using the Ilizarov external fixation and the Masquelet technique. *Genij Ortopedii*. 2020; 26(2):216-221. doi: 10.18019/1028-4427-2020-26-2-216-22.
39. Wang Y, Lu J, Liu Y. Skeletal Muscle Regeneration in Cardiotoxin-Induced Muscle Injury Models. *Int J Mol Sci*. 2022;23(21):13380. doi: 10.3390/ijms232113380.
40. Broer T, Tsintolas N, Purkey K, et al. Engineered myovascular tissues for studies of endothelial/satellite cell interactions. *Acta Biomater*. 2024;188:65-78. doi: 10.1016/j.actbio.2024.09.020.
41. Hendrickse P, Degens H. The role of the microcirculation in muscle function and plasticity. *J Muscle Res Cell Motil*. 2019;40(2):127-140. doi: 10.1007/s10974-019-09520-2.
42. Yang X, Xue P, Chen H, et al. Denervation drives skeletal muscle atrophy and induces mitochondrial dysfunction, mitophagy and apoptosis via miR-142a-5p/MFN1 axis. *Theranostics*. 2020;10(3):1415-1432. doi: 10.7150/thno.40857.
43. Zhang L, Li M, Wang W, et al. Celecoxib alleviates denervation-induced muscle atrophy by suppressing inflammation and oxidative stress and improving microcirculation. *Biochem Pharmacol*. 2022;203:115186. doi: 10.1016/j.bcp.2022.115186.

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