ORIGINAL ARTICLE

Nr 2025;15 (2):155-161

Morphometric Analysis of Flexor Carpi Radialis and Flexor Digitorum Muscles in Normal Young Wistar Rats

Marcílio Coelho Ferreira, Castrine Lemos Barbosa, Diego Mendes Xavier, Thaís Peixoto Gaiad, Murilo Xavier Oliveira, Ana Paula Santos

Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Diamantina, Brazil

CORRESPONDING AUTHOR:
Diego Mendes Xavier
Department of Physiotherapy
Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM)
Rodovia MG 367
5000 Alto da Jacubá
39100-000 Diamantina (MG), Brazil
E-mail: diegomendesxav@gmail.com

DOI:
10.32098/mltj.02.2025.05

LEVEL OF EVIDENCE: N/A (animal study)

SUMMARY
Normative muscle fiber morphometrics serve as a reference to assess injuries and the response to different treatments and as well as physiological adjustments that occur in sports or during the aging process. Animal models using rats are common, but data on muscle morphometrics are scarce. The purpose of this study was to determine the morphometrics of the flexor carpi radialis (FCR) and flexor digitorum (FD) muscles in normal Wistar rats. Experimental study approved by the animal use ethics committee (n. 030/2016). Eight female Wistar rats eight weeks of age were used. The muscles were fixed, dehydrated, and embedded in Paraplast®. The cuts were stained with hematoxylin and eosin and photomicrographed. The analysis was performed using the ImageJ program, with the determination of the area, perimeter, Feret's diameter, and number of muscle fibers in 10% of the frames (magnification: 40 x). Descriptive data analysis was performed. The average number of muscle fibers was 437 ± 138 for the FCR and 546 ± 232 for FD. Average perimeter, diameter, and area of the FCR muscle fibers were 74.42 ± 12.62 µm, 27.35 ± 5.26 µm, and 387.66 + 133.57 µm^2, respectively. These figures were 76.73 ± 14.84 µm, 28.62 ± 6.23 µm, and 411.22 ± 155.50 µm^2, respectively, for the FD muscle fibers. These results are important, especially for experimental studies involving the thoracic limb of rats for comparisons to normative reference values.

KEY WORDS
Histology; skeletal muscle; rat Wistar; reference values.

INTRODUCTION

Skeletal muscle is susceptible to injury (1) and can be affected by damage to other tissues, such as bones and nerves (2, 3). Disease, aging, training, and treatments can also impact the structure of skeletal muscle (4-6).

Animal models are extensively used to understand various dysfunctions of human body systems and explore treatment options (7, 8). Animal experimentation plays a crucial role in advancing biomedical research and discovering new therapeutic approaches. Rats and mice are the most commonly used models in scientific research (8). Particularly, Wistar rats are used in scientific studies due to their genetic similarity to humans (9, 10).

The importance of morphometric image analysis in biomedical research is well established (11). Standardization studies are essential to understanding normal tissue characteristics before investigating abnormalities and for potentially reducing the number of animals used in scientific studies, as guided by animal use ethics committees (8). Quantitative analysis of muscle fibers offers precise, rigorous determinations of fiber size and number and is critical for studying the aging process, diseased muscle, as well as responses to training and treatments (12, 13).

Most studies on the microscopic anatomy of skeletal muscles focus on the pelvic limb muscles of rats, which are frequently used in experimental models (14, 15). Normative morphometric data for the flexor digitorum (FD) and flexor carpi radialis (FCR) muscles in Wistar rats are lacking. Information on the weight and fiber types of the FD and FCR muscles has been detailed in studies involving median nerve injury models

(16-18). One study provided morphometric data on muscle fiber morphology at the neuromuscular junction of the FD muscle in Wistar rats (19).

The FD and FCR muscles are responsible for movements assessed in functional tests of the thoracic limb of rats, such as the grasping test (20), which is used to analyze grasping ability, and the staircase test (21), which encompasses motor skills, reach, and grasping. Such tests are employed in research on peripheral and central nervous system injuries (18, 19).

The aim of the present study was to determine morphometrics of the FCR and FD muscle fibers in normal young Wistar rats to provide normative reference data.

METHODS

This study adhered to the guidelines of Brazilian College of Animal Experimentation and protocols approved by the Regional Council of Veterinary Medicine. The study received approval from the animal use ethics committee of Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM) under protocol no. 030/2016 – date of approval: August 3, 2016.

Eight female Wistar rats eight weeks of age and weighing approximately 220 grams each were used. The animals were housed in plastic cages (maximum of four rats per cage) with free access to food and water and kept in climate-controlled rooms with a temperature of 22-23 °C, a 12-hour light/dark cycle, and air ventilation. The cages were lined with sufficient bedding to ensure that the animals remained warm.

Following standard care procedures, including weight verification and anesthesia (ketamine 80 mg/kg and xylazine 15 mg/kg – 0.15 ml/100 g of body weight – administered intraperitoneally) and confirmation of the anesthetic effect, the rats underwent surgery to excise the FCR and FD muscles. An incision was made through the skin and fascia of the forearm. The FCR and FD muscles were fixed *in situ*, removed (**figure 1**), and preserved in a 4% paraformaldehyde solution for 24 hours.

The samples were subjected to a series of increasing ethanol concentrations (70% to 100%) for dehydration and xylene for clearing and then embedded in Paraplast® (Leica, Germany). Rectangular blocks measuring 2 × 3 cm were prepared and sectioned at a thickness of 5 µm using a microtome to produce histological slides. The slides were placed in an oven at 60 °C for two hours and subsequently stained with hematoxylin and eosin.

After staining, sequential images of the slides were captured using with an Axio CAM ERc5s camera coupled to a Primo Star optical microscope (Zeiss) (magnification: 40 x). Images were acquired with the AxioVision LE software, version 4.8. Image analysis was performed using the ImageJ software, from which data on area, perimeter, fiber count, and Feret's diameter were obtained for 10% of the fields. The number of fibers was determined by counting complete intact fibers with a normal polygonal appearance (**figure 2**) and recording fibers in the upper left margins of each field. Area, perimeter, and diameter measurements were calculated by the software following precise manual delineation of each fiber (**figure 2**). Statistical analysis was performed using the Minitab 15 software and involved descriptive statistics (mean, standard deviation, minimum, and maximum values).

RESULTS

The FCR and FD muscle fibers exhibit a typical pattern, with a polygonal shape, nuclei always in a peripheral posi-



Figure 1. Removal of flexor carpi radialis muscle from Wistar rat.

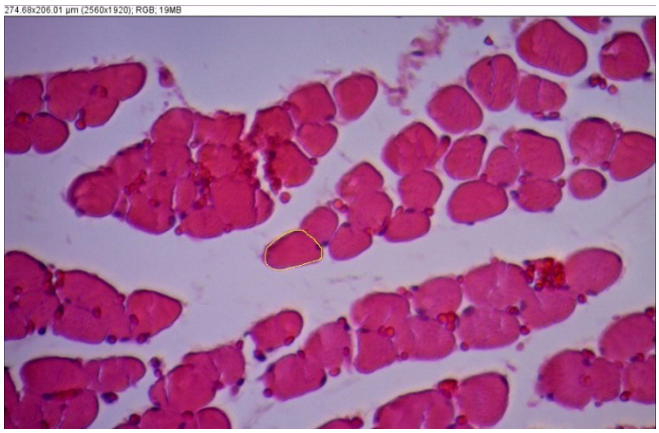


Figure 2. Photomicrograph of cross-section of flexor digitorum muscle of Wistar rat exemplifying delimitation of muscle fiber with polygonal appearance (yellow line).
Staining: hematoxylin and eosin. Magnification: 40X.

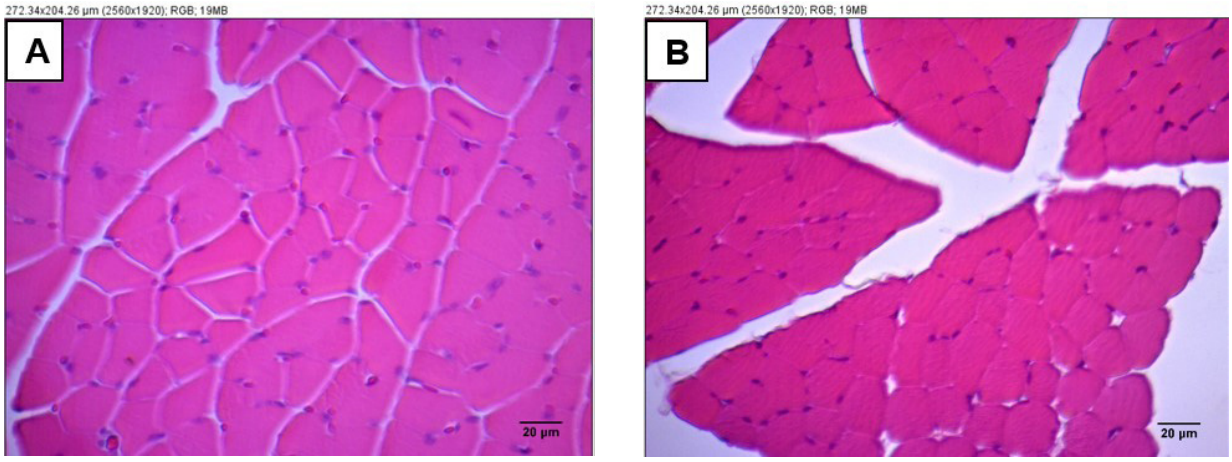


Figure 3. Photomicrograph of cross-section of belly of flexor digitorum (**A**) and flexor carpi radialis (**B**) muscles of Wistar rat. Staining: hematoxylin and eosin. Magnification: 40X

Table I. Morphometric data of flexor digitorum muscle fibers of young female Wistar rats.

Flexor digitorum	Mean ± standard deviation	Minimum	Maximum
Number of fibers*	545.70 ± 231.90	253.00	944.00
Perimeter (µm)	76.73 ± 14.84	38.90	136.44
Feret's diameter (µm)	28.62 ± 6.23	14.96	59.90
Area (µm2)	411.22 ± 155.50	96.47	1,093.45

*Referring to 10% of frames.

Table II. Morphometric data of flexor carpi radialis muscle fibers of young female Wistar rats.

Flexor carpi radialis	Mean ± standard deviation	Minimum	Maximum
Number of fibers*	436.70 ± 138.40	254.00	678.00
Perimeter (µm)	74.42 ± 12.62	40.41	153.03
Feret's diameter (µm)	27.35 ± 5.26	14.49	54.46
Area (µm2)	387.66 ± 133.57	113.70	1,765.28

*Referring to 10% of frames.

tion, and little connective tissue, which are characteristics of normal muscle tissue (**figure 3**).

The number of muscle fibers, perimeter, diameter, and area of the FD and FCR muscle fibers are detailed in **tables I** and **II**, respectively.

DISCUSSION

This study presents the first histomorphometric information on the FCR and FD muscles of normal young Wistar rats, which is a lineage and age commonly used in experimental studies. Investigating these muscles is crucial due

to their importance in models of median nerve injury, where the pursuit of successful regeneration translates into functional recovery associated with muscle integrity (22), and in repetitive strain injury models commonly performed with rats (23, 24). The lack of descriptions of thoracic limb muscles in rats makes the study of these muscles necessary.

Morphometrics was one of the investigation methods used in this study, which enables the quantitative objective description of structures, revealing minimal morphological differences. Morphometrics is important in the health field for the study of diseases (diagnosis and prognosis) and treatments as well as the determination of normative values (25-28).

Feret's diameter was chosen because it is widely used in studies analyzing muscle tissue (29, 30), providing reliable results with regards to the variation in fiber size, Feret's diameter is also minimally affected by the cutting angle, which reduces the occurrence of measurement errors (31, 32).

Manual morphometrics is time consuming, challenging to perform accurately, and tedious, predisposing the observer to fatigue, and is subject to many sources of error (33, 34). Although precise and less time consuming (33, 35), fully automated computer image analysis systems require specialized equipment, computers with ample memory, and sophisticated software, making the process expensive and sometimes difficult to implement (34, 36). Moreover, fully automated morphometrics can convert uncorrected image interpretation errors into false data (36, 37). Therefore, semi-automatic methods that enable user interaction are currently considered the gold standard for specimen morphometrics (38), which was the muscle analysis method used in this study.

Few studies on the FCR and FD muscles are found in the literature, independently of the focus, such as differences in muscle fibers between the sexes (39), different ages (40), the analysis of normal muscle fiber types (41), healthy and diseased fibers (42, 43), disuse atrophy (44), the effect of exercise (45), and the effect of low-intensity laser therapy on injured muscles (46).

Boreham *et al.* (1988) (47) performed a qualitative histological description of the FD in Sprague-Dawley rats, analyzing the effect of age and nutritional restriction on thoracic and pelvic limb muscles. The extensor digitorum longus and soleus muscles were analyzed morphometrically, but the only information on the FD was a cross-sectional image of the muscle of an elderly animal, which revealed less cellular senescence compared to the soleus muscle.

The FD muscle is studied in repetitive task models (grasping), with analyses focused on inflammatory processes, such as changes in connective tissue growth factor, type I collagen, and transforming growth factor beta 1. Papalia *et al.* (2012) (48) analyzed the flexor muscle of the fingers in Wistar rats after neurotmesis followed by suture, using cross-sections stained with toluidine blue with no characteristic signs of atrophy and no control group, assessing function through the grasping test.

Jiang *et al.* (2016) (49) analyzed the electrical activity and morphometrics of the flexor digitorum superficialis (FDS) muscle in a brachial plexus injury model with nerve grafts in Sprague-Dawley rats. The variable of interest was the cross-sectional area of the muscle fiber. Four weeks after surgery, the FDS muscles of the experimental groups exhibited significant atrophy, with gradual recovery over time. The group that received a contralateral C7 root graft exhibited greater recovery of muscle fiber area compared to the group with intercostal nerve graft, both of which were inferior to the uninjured control. Guzzini *et al.* (2008) (50) studied the effects of 30 days of denervation on muscle trophism and the distribution of acetylcholine receptors. After denervation, severe atrophy was found in the FDS muscle, as confirmed by the reduction in weight.

Bertelli *et al.* (1994) (51) studied the FCR muscle after median nerve neurotmesis in Sprague-Dawley rats, finding a significant reduction in the weight of the muscle. In normal rats, the weight of the FCR muscle was described as 80 ± 5 grams (48, 49). The authors also investigated the fiber organization of the FCR using ATPase analysis, describing a predominance of fast-twitch (type II) fibers. Muscle fiber types were analyzed in C57BL/6J mice, with a higher percentage of fast/oxidative/glycolytic-coarse fibers described in the FCR and FD muscles. Muscle diameter ranged from 27 to 38 µm in the FCR and 25 to 31 µm in the FD. In our study, average fiber diameter was 27.35 µm in the FCR and 28.61 µm in the FD (47-49).

Pan *et al.* (2016) (52) analyzed muscle fiber types and endplates in the intrinsic muscles of the thoracic limb of rats using immunofluorescence. Except for interosseous muscles, which only had slow fibers, the other intrinsic muscles exhibited hybrid fibers, with a predominance of fast-twitch fibers.

Limitations and originality

The present study presents several noteworthy limitations. First, the absence of muscle mass measurements for the FCR and FD muscles hinders the ability to perform a comparative analysis of muscle trophism. Furthermore, the lack of classification and detailed analysis of muscle fiber types

limits our understanding of specific muscle adaptations. The use of observational methods without incorporating advanced techniques, such as immunofluorescence, and the absence of a properly matched control group further constrain the interpretation of the findings. Additionally, variability in experimental models and injury induction methods introduces inconsistencies in the outcomes, making comparisons with other studies more challenging. The analysis was also limited to a single time point, with no longitudinal follow-up to assess changes over time. While the manual morphometric methods employed are precise, they are less efficient compared to automated techniques. Finally, the results obtained in young Wistar rats may not be fully generalizable to other strains, age groups, or species, limiting the broader applicability of the findings. However, the originality of this study lies in its provision of detailed morphometric data on the FCR and FD muscles in young Wistar rats, which, to date, has been absent from the literature. These data serve as normative references for future studies exploring muscle regeneration, the effects of treatments, and adaptations to injury, and are applicable to experimental models involving both the peripheral nervous system and muscular injuries.

Future studies should employ more advanced techniques, rigorous control, and longitudinal analyses to achieve a more comprehensive understanding of the FCR and FD muscles under different experimental conditions. To achieve a more comprehensive understanding of specific muscle adaptations and enhance the generalizability of the results, future studies should incorporate advanced techniques, such as immunofluorescence, conduct longitudinal analyses, and include rigorous control groups. Additionally, exploring different strains, ages, and species will be crucial for validating and expanding the findings of this study.

Histomorphometric analysis of the FCR and FD muscles can contribute knowledge for models of peripheral and central nervous system injuries. To obtain more detailed data on functional recovery, a histomorphometric analysis of the FCR and FD muscles can be performed with knowledge of normative reference values.

Parte inferior do formulário

CONCLUSIONS

This study provides the first detailed histomorphometric information on the FCR and FD muscles in young normal Wistar rats, making a significant contribution to the field of muscle morphometrics. The study demonstrates that morphometrics can be a valuable method for quantitatively and objectively describing muscle structures, highlighting the importance of semiautomatic techniques to minimize errors and enhance accuracy. Histomorphometric analysis of the FCR and FD muscles can deepen the understanding of models of peripheral and central nervous system injuries and the determination of normative reference values is essential for strengthening scientific discussions.

FUNDINGS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) - APQ-00062-18This work was supported by the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG).

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

MCF: conceptualization, methodology, investigation, formal analysis, writing - original draft. CLB: methodology, investigation, writing - original draft. DMX: formal analysis, writing - original draft. TPG: supervision, writing - review & editing. MXO: supervision, writing - review & editing, writing - original draft. APS: supervision, writing- review & editing.

ACKNOWLEDGEMENTS

We would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (National Council for Scientific and Technological Development) (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Coordination for the Improvement of Higher Education Personnel) (CAPES), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Research Support Foundation for the State of Minas Gerais) (FAPEMIG) for support and grants

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. Edouard P, Azzam A, Wiemi J, et al. Traumatic muscle injury. Nat Rev Dis Primers. 2023;9(1):56. doi: 10.1038/s41572-023-00424-0.
2. Mehrotra P, Kwon J, Xie X, et al. Skeletal muscle reprogramming enhances reinnervation after peripheral nerve injury. Res Sq. 2024;5:463557. doi: 10.21203/rs.3.rs-1234567/v1.
3. Willey MC, Lacheta L, Ramachandran S, et al. Substantial loss of skeletal muscle mass occurs after femoral fragility fracture. J Bone Joint Surg Am. 2023;105(22):1777-85. doi: 10.2106/JBJS.23.00234.
4. Chen YL, Zhang J, Liu Y, et al. Physical exercise attenuates age-related muscle atrophy and exhibits anti-aging effects via the adiponectin receptor 1 signalling. J Cachexia Sarcopenia Muscle. 2023;14(4):1789-801. doi: 10.1002/jcsm.13330.
5. Heitman K, Alexander M, Faul C. Skeletal muscle injury in chronic kidney disease-from histologic changes to molecular mechanisms and to novel therapies. Int J Mol Sci. 2024;25(10):5117. doi: 10.3390/ijms25105117.
6. Kimira Y, Nakamura T, Sakurai K, et al. Preventive effects of collagen-derived dipeptide prolyl-hydroxyproline against dexamethasone-induced muscle atrophy in mouse C2C12 skeletal myotubes. Biomolecules. 2023;13(11):1617. doi: 10.3390/biom13111617.
7. Meassil AJK, Oliveira CS, Oliveira GP, et al. Effect of Therapeutic Ultrasound on the Recovery of the Tibialis Anterior Muscle in Remobilized Wistar Rats after a Period of Immobilization: An Analysis Using the Pathological Index. Muscles Ligaments Tendons J. 2024;14 (2):278-83. doi: 10.32098/mltj.02.2024.06.
8. Kiani AK, Haider S, Ramesh S. Ethical considerations regarding animal experimentation. J Prev Med Hyg. 2022;63(2 Suppl 3) doi: 10.15167/2421-4248/jpmh2022.63.2s3.3171.
9. Ferreira GV, Souza R, Oliveira M, et al. Effect of using Apevitin BC® in rats (Rattus norvegicus). Braz J Anim Environ Res. 2024;7(1):777-90. doi: 10.5935/2236-5473.20240077.
10. Nakanishi S, Nishioka N, Sasaki K, et al. Slcoutbred rats show close genetic similarity with F344 inbred rats. Exp Anim. 2015;64(1):25-9. doi: 10.1538/expanim.64.25.
11. Merhar V, Naicker T. Morphometric image analysis and its applications in biomedicine using different microscopy modes. In: Shapiro L, editor. Microscopy techniques for biomedical education and healthcare practice. Biomed Vis. Cham: Springer; 2023. p. 2. doi: 10.1007/978-3-030-37991-6_2.
12. Gropp KE. Skeletal Muscle Toolbox. Toxicol Pathol. 2017;45(7):939-42. doi: 10.1177/0192623317731336.
13. Reyes-Fernandez PC, Coudray S, Albalat A, et al. Automated image-analysis method for the quantification of fiber morphometry and fiber type population in human skeletal muscle. Skelet Muscle. 2019;9(1):15. doi: 10.1186/s13395-019-0276-6.
14. Goncharuk O, Vovk V, Palchykova S, et al. Quantitative histological assessment of skeletal muscle hypotrophy after neurotomy and sciatic nerve repair in rats. Georgian Med News. 2021;313:169-72. doi: 10.46848/gmn.2021.313.169.
15. Tibúrcio FC, Peres F, Valero M, et al. Neuroregeneration and immune response after neurorrhaphy are improved with the use of heterologous fibrin biopolymer in addition to suture repair alone. Muscle Nerve. 2023;67(6):522-36. doi: 10.1002/mus.27795.
16. Bertelli JA, Mira JC, Dallalana A, et al. The rat brachial plexus and its terminal branches: an experimental model for the study of peripheral nerve regeneration. Microsurgery. 1995;16(2):77-85. doi: 10.1002/micr.1920160203.
17. Bertelli JA, Mira JC, Dallalana A, et al. Muscle fiber type reorganization and behavioral functional recovery of rat median nerve repair with vascularized or conventional nerve grafts. Restor Neurol Neurosci. 1996;10(1):5-12. doi: 10.3233/RNN-1996-101-102.
18. Casal D, Lima S, Cunha J, et al. Functional and physiological methods of evaluating median nerve regeneration in the rat. J Vis Exp. 2020;(158):59767. doi: 10.3791/59767.
19. Estrada-Bonilla YC, García-López A, Cruz-Medina E, et al. Compensatory neuromuscular junction adaptations of forelimb muscles in focal cortical ischemia in rats. Brain Behav. 2020;10(3). doi: 10.1002/brb3.1472.
20. Lauer H, Verhoeff C, Kropf S, et al. The Grasping Test revisited: A systematic review of functional recovery in rat models of median nerve injury. Biomedicines. 2022;10(8):1878. doi: 10.3390/biomedicines10081878.
21. Montoya CP, Wang G, McDonald W, et al. The "staircase test": a measure of independent forelimb reaching and grasping abilities in rats. J Neurosci Methods. 1991;36(2-3):219-28. doi: 10.1016/0165-0270(91)90022-A.
22. Udina E, Ceballos D, Gold BG, Navarro X. Electrical stimulation of intact peripheral sensory axons in rats promotes outgrowth of their central projections. Exp Neurol. 2011;233(1):100-11. doi: 10.1016/j.expneurol.2011.09.001.
23. Boreham CA, Watt PW, Williams PE, Merry BJ, Goldspink G, Goldspink DF. Effects of ageing and chronic dietary restriction on the morphology of fast and slow muscles of the rat. J Anat. 1988;157:111-25. doi: 10.1002/j.1469-7580.1988.tb02755.x.
24. Abdelmagid SM, Barr A, Rico M, et al. Performance of repetitive tasks induces decreased grip strength and increased fibrogenic proteins in skeletal muscle: role of force and inflammation. PLoS One. 2012;7(5). doi: 10.1371/journal.pone.0038359.
25. Gilmore SA. Spinal nerve root degeneration in aging laboratory rats: a light microscopic study. Anat Rec. 1972;174(2):251-7. doi: 10.1002/ar.1091740207.
26. Adhami H, Sawatzky KH, Hornung J. Morphometric studies on the relationship between myelin sheath and axon area in the sciatic nerve of adult rats. Anat Anz. 1976;140(1-2):52-61. doi: 10.1016/S0003-2778(76)80007-5.
27. Reith A. Relevance of morphometry in pathology: with special emphasis on cancer-related studies. Acta Anat. 1981;111:124. doi: 10.1159/000145895.
28. Raimondo S, Fornaro M, Di Scipio F, Ronchi G, Giacobini-Robecchi MG, Geuna S. Methods and protocols in peripheral nerve regeneration experimental research: part II-morphological techniques. Int Rev Neurobiol. 2009;87:81-103. doi: 10.1016/S0074-7742(09)87005-8.

29. Laghi V, Ricci V, De Santa F, Torcinaro A. A User-Friendly Approach for Routine Histopathological and Morphometric Analysis of Skeletal Muscle Using CellProfiler Software. Diagnostics (Basel). 2022;12(3):561. doi: 10.3390/diagnostics12030561.
30. Zhang H, Wang F, Pang X, et al. Decreased expression of H19/miR-675 ameliorates muscle atrophy by regulating the IGF1R/Akt/FoxO signaling pathway. Mol Med. 2023;29(1):78. doi: 10.1186/s10020-023-00683-w.
31. Siemionow M, Budzynska K, Zalants K, et al. Amelioration of Morphological Pathology in Cardiac, Respiratory, and Skeletal Muscles Following Intraosseous Administration of Human Dystrophin Expressing Chimeric (DEC) Cells in Duchenne Muscular Dystrophy Model. Biomedicines. 2024;12(3):586. doi: 10.3390/biomedicines12030586 2024.
32. Hegarty PV, Hooper AC. Sarcomere length and fibre diameter distributions in four different mouse skeletal muscles. J Anat. 1971;110(Pt 2):249-57.
33. Vita G, Santoro M, Trombetta G, Leonardi L, Messina C. A computer-assisted automatic method for myelinated nerve fiber morphometry. Acta Neurol Scand. 1992;85(1):18-22. doi: 10.1111/j.1600-0404.1992.tb05462.x.
34. Silva APD, Jordão CER, Fazan VPS. Peripheral nerve morphometry: comparison between manual and semi-automated methods in the analysis of a small nerve. J Neurosci Methods. 2007;159(1):153-7. doi: 10.1016/j.jneumeth.2006.07.015.
35. Romero E, Cuisenaire O, Denef JF, Delbeke J, Macq B, Veraart C. Automatic morphometry of nerve histological sections. J Neurosci Methods. 2000;97(2):111-22. doi: 10.1016/S0165-0270(00)00304-6.
36. Urso-Baiarda F, Grobbelaar AO. Practical nerve morphometry. J Neurosci Methods. 2006;156(1-2):333-41. doi: 10.1016/j.jneumeth.2006.02.013.
37. Zimmerman R, Karnes JL, O'Brien PC, Dyck PJ. Imaging system for nerve and fiber tract morphometry: components, approaches, performance, and results. J Neuropathol Exp Neurol. 1980;39(4):409-19. doi: 10.1097/00005072-198007000-00004.
38. Bilego Neto APC, Silveira FB, Silva GAR, Sanada LS, Fazan VPS. Reproducibility in nerve morphometry: comparison between methods and among observers. Biomed Res Int. 2013;2013:682849. doi: 10.1155/2013/682849.
39. Mierzejewska-Krzyżowska B, Drzymała-Celichowska H, Bukowska D, Celichowski J. Gender differences in morphometric properties of muscle fibres measured on cross-sections of rat hindlimb muscles. Anat Histol Embryol. 2011;41(2):122-9. doi: 10.1111/j.1439-0264.2010.01084.x.
40. Alnaqeeb MA, Goldspink G. Changes in fibre type, number and diameter in developing and ageing skeletal muscle. J Anat. 1987;153:31-45. doi: 10.1111/j.1469-7580.1987.tb02341.x.
41. Pette D, Peuker H, Staron RS. The impact of biochemical methods for single muscle fibre analysis. Acta Physiol Scand. 1999;166(3):261-77. doi: 10.1046/j.1365-201x.1999.00597.x.
42. Aughsteen AA, Khair AM, Suleiman AA. Quantitative morphometric study of the skeletal muscles of normal and streptozotocin-diabetic rats. JOP. 2006;7(4):382-9. doi: 10.6092/1590-8577/2015/07/04/382-389.
43. Yun PH, DeLuca S, Robinson D, et al. Tenforde Radial versus Combined Shockwave Therapy in the Management of Proximal Hamstring Tendinopathy: Similar Functional Outcomes in Running Cohort. Muscles Ligaments Tendons J. 2021;11 (4):742-8. doi: 10.32098/mltj.04.2021.18.
44. Itai Y, Kariya Y, Hoshino Y. Morphological changes in rat hindlimb muscle fibres during recovery from disuse atrophy. Acta Physiol Scand. 2004 Nov;181(2):217-24. doi: 10.1046/j.1365-201x.2004.01371.x.
45. Mattos de Brito MK, Camargo Filho JC, Vanderlei LC, Tarumoto MH, Dal Pai V, Giacometti JA. Dimensões geométricas das fibras do músculo sóleo de ratos exercitados em esteira rolante: a importância da análise por meio de imagens digitalizadas. Rev Bras Med Esporte. 2006;12(2):103-7. doi: 10.1590/S1517-86922006000200008.
46. Costardi CHZ, Tamachiro C, Júnior IE, Gomes AC. Effects of low-energy laser (670nm) irradiation after muscle crush injury in rats. Fisioter Mov. 2008;21(2):21-30. doi: 10.1590/S0103-51502008000200003.
47. Boreham CA, Watt PW, Williams PE, Merry BJ, Goldspink G, Goldspink DF. Effects of ageing and chronic dietary restriction on the morphology of fast and slow muscles of the rat. J Anat. 1988;157:111-25. doi: 10.1111/j.1469-7580.1988.tb02755.x.
48. Papalia I, Ronchi G, Muratori L, Mazzucco A, Magaudda L, Geuna S. Direct muscle neurotization after end-to-end and end-to-side neurorrhaphy: an experimental study in the rat forelimb model. Neural Regen Res. 2012;7(29):2273-8. doi: 10.3969/j.issn.1673-5374.2012.29.001.
49. Jiang Y, Wang L, Lao J, Zhao X. Comparative study of intercostal nerve transfer to lower trunk and contralateral C7 root transfer in repair of total brachial plexus injury in rats. J Plast Reconstr Aesthet Surg. 2016;69(5):623-8. doi: 10.1016/j.bjps.2015.12.008.
50. Guzzini M, Raffa S, Geuna S, Net al. Denervation-related changes in acetylcholine receptor density and distribution in the rat flexor digitorum sublimis muscle. Ital J Anat Embryol. 2008;113(4):209-16. doi: 10.1179/112073208X301660.
51. Bertelli JA, Mira JC. The grasping test: a simple behavioral method for objective quantitative assessment of peripheral nerve regeneration in the rat. J Neurosci Methods. 1995;58(1-2):151-5. doi: 10.1016/0165-0270(94)00172-Y.
52. Pan F, Mi J, Zhang Y, Pan X, Rui Y. Muscle fiber types composition and type identified endplate morphology of forepaw intrinsic muscles in the rat. J Muscle Res Cell Motil. 2016;37(3):95-100. doi: 10.1007/s10974-016-9441-8.