2024
11(4), 674

Chemical Biology
LETTERS

Dynamics of neuromuscular junction degeneration after denervation: Mini review

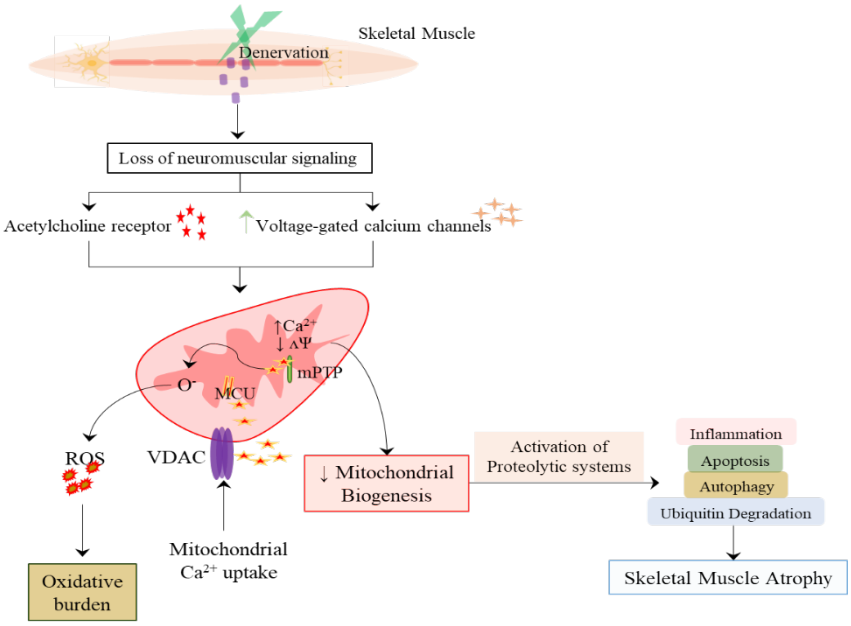
Aarti Yadav, Rajesh Dabur*

Department of Biochemistry, Maharshi Dayanand University, Rohtak, Haryana-124001, India

Submitted on: 24-Mar-2024, Accepted and Published on: 12-Jul-2024

Review Article

ABSTRACT



Nerve pulses open the calcium channel on the sarcolemma, allowing calcium and sodium ions to enter the sarcoplasm. However, denervation changes the resting membrane potential to depolarize myofibers within hours with increased fibrillation activity. Increased influx of Ca^{2+} activates the dehydrogenases of Kreb's cycle, uncouples the oxidative phosphorylation, and increases the mitochondrial permeability through the opening of the permeability transition pore (PTP) and mitochondrial calcium uniporter (MCU) complex. Therefore, levels of free oxygen and nitrogen radicals are upregulated, to increase the inflammation and the main mechanisms including apoptosis, autophagy, and ubiquitin-proteasomal degradation of skeletal muscle atrophy. Increased Ca^{2+} pool also declines the mitochondrial ATP, activating AMP-activated protein kinase (AMPK) followed by forkhead box protein O3 (FoxO3) expression and increasing the incidence of mitophagy and ubiquitination of muscle proteins. Therefore, this current mini-review offers a comprehensive examination of denervation-induced muscle atrophy, shedding light on the intricate nature of regulatory mechanisms in cases of sciatic injury.

Keywords: Nerve pulses, Denervation, Ca^{2+} signaling, Mitochondria permeability, Inflammation, Skeletal muscle atrophy

INTRODUCTION

Skeletal muscle (SkM) is the most prevalent kind of tissue in vertebrates and is associated with strength, stamina, and endurance; as a result, it has the greatest rate of glucose absorption in the body.[1] Skeletal muscle atrophy is characterized by loss of SkM mass due to excessive protein breakdown, which reduces the cross-sectional area of myofibers and muscular strength. Various pathological states, including sarcopenia, denervation, cachexia, immobilization, malnutrition, and metabolic disorders, are associated with SkM atrophy. Despite various pathophysiologies, very downstream events of SkM atrophy are common, i.e., activation of proteolytic systems and removal of contractile proteins and organelles. However, upstream stimulators of SkM may be different or common depending on the pathophysiological state.[2] As a result, the molecular signaling pathways underlying SkM atrophy may change depending on the circumstances.

In the SkM, neurotransmission is maintained through neuromuscular junctions (NMJ) present on the SkM to maintain muscle tone and function through motor neurons.[3] Generally, the NMJ synapse is characterized by a synaptic cleft between the presynaptic and postsynaptic membranes, spherical vesicles, and a thick postsynaptic membrane containing specialized receptors.[4] A nerve action potential is propagated through motor neurons and releases acetylcholine (Ach) in the synaptic cleft. It has been reported that endplate potential is critical for muscle contraction and varies up to 50mV in magnitude to ensure that the muscle

*Corresponding Author: Prof. Rajesh Dabur, Department of Biochemistry, Maharishi Dayanand University, Rohtak, Haryana-124001, India.
E-Mail: rajeshdabur@yahoo.com ; Tel: +91-1262-393070

URN:NBN:sciencein.cbl.2024.v11.674

https://pubs.thesciencein.org/cbl

fiber reaches the threshold for generation of the 30mV action potential. However, the difference between the amplitude of action potential and the threshold is called the "safety factor", which acts as a buffer for muscle contraction even under less ideal conditions and releases Ach. Release Ach binds to nicotinic acetylcholine receptors (nAchR, prototypical cation-selective, ligand-gated ion channels receptors) present on the postsynaptic membrane.[4,5] These nAchR channels increase the permeability of Na^+ and K^+ specifically and initiate depolarization of the postsynaptic membrane of 10 msec duration to initiate the excitation-contraction process.[6,7] Additionally, action potentials in myofiber activate muscarinic acetylcholine receptor (mAchR) 1,3, and 5, which lead to Ca^{2+} release from the sarcoplasmic reticulum (SR) and trigger myofiber contraction.[7,8] Normally, the membrane potential of SkM cells is higher than -80 mV. The channel opened by decreased Ach has more permeability to Na^+ and K^+ hence, the membrane potential would move toward 0 mV, and no potential change is recorded.[9,10] However, the end plate potential is reversed if the membrane potential reaches +30 mV.[11] Released Achs in the synaptic cleft has been hydrolyzed by acetylcholinesterase (AchE) with a rate of 600,000 molecules of Ach/min to end up with depolarization.[12] Hence, polarization and depolarization of the myofiber membrane is the fundamental regulator of muscle contraction and relaxation through quick shifts in myoplasmic Ca^{2+} levels controlled by the sarcoplasmic reticulum (SR).[13]

In addition to Ca^{2+}, SkM also needs adenosine triphosphate (ATP) as a source of energy, hence, heavily relies on mitochondria being the primary source of ATP production during contractions and relaxations. Furthermore, mitochondrial Ca^{2+} levels influence mitochondrial dynamics, function, and metabolism in SkM.[14] Thus, for proper work of SkM, precise regulation of Ca^{2+} signaling and ROS generation is a basic prerequisite.

Sciatic Nerve Injury and Ca^{2+} Signaling

Sciatic nerve injury or neurodegeneration due to sarcopenia, metabolic disorders, or any other disease condition interrupts the action potential propagated through motor neurons **(Figure 1)** and the release of Ach in the synaptic cleft.[15] Lack of Ach deactivates muscarinic acetylcholine receptor (M) 1,3 and 5 as well as voltage-gated calcium channels (VGCC), nAchR, and muscle fiber cells remain in depolarization state to maintain a high concentration of intracellular Ca^{2+} levels due to increased permeability of cellular membranes.[16] Recently, elevated expression of the connexin (Cx) 43 and 45 gap junction hemichannels (HCs) and purinergic receptors (P2X7Rs) were observed in denervated myofibers known to be permeable for Ca^{2+} flux. Further, Cx43/Cx45 HCs were reported to leak ATP from intracellular to extracellular spaces, which can activate P2X7Rs and promote Ca^{2+} influx further.[17] Moreover, high levels

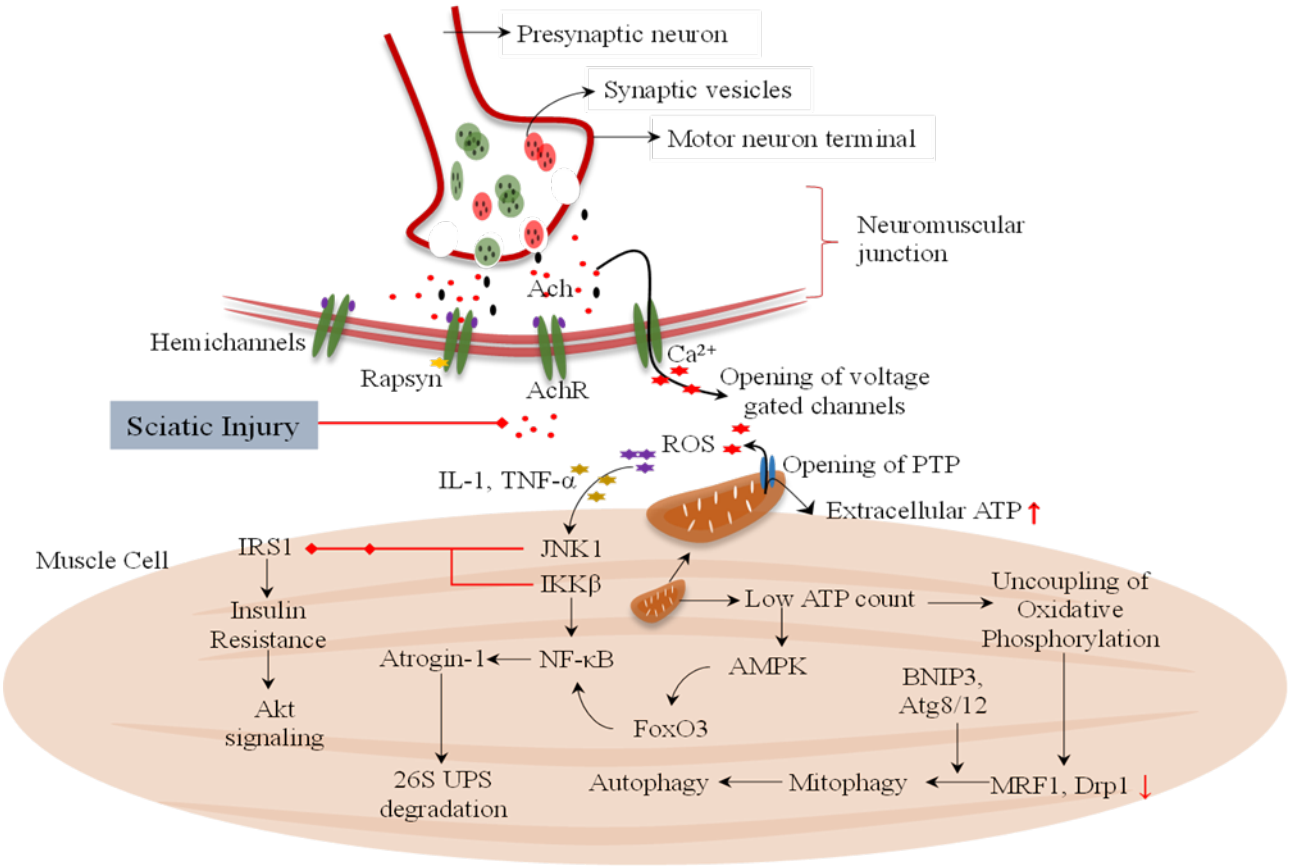


Figure 1: Systematic representation of alerted signaling of protein catabolism after sciatic injury in skeletal muscles.

of free extracellular Ca^{2+} [~10,000-fold] may also contribute to excessive Ca^{2+} entry in the cytosol.[18]

Ca^{2+} signaling within cells is modulated, buffered, and sensed by mitochondria.[19] After denervation, SkM cells remain depolarized with elevated cytosolic Ca^{2+} levels; therefore, mitochondria take up Ca^{2+} to avoid cytosolic Ca^{2+} overload. Also, depolarized muscle cells express more of the MCU complex, which is essential for controlling Ca^{2+} levels in the mitochondrial matrix. Moreover, Ca^{2+} overload in the mitochondrial matrix opens the mitochondrial PTPs, which further increase Ca^{2+} levels and activate the mitochondrial death pathway. Increased PTP opening declines the resting membrane potential, and ATP synthesis, and uncouples the oxidative phosphorylation.[20] Additionally, elevated levels of Ca^{2+} inside the mitochondrial matrix keep hyperactivated Kreb cycle's Ca^{2+}-sensitive enzymes and F1/F0 ATPase.[21] Hence, it releases free radicals due to the uncoupling of the electron transport chain, which activates PTP to enhance ROS production further. Increased ROS also oxidize ryanodine receptor 1 (RyR1) Ca^{2+} release channels of SR that allow Ca^{2+} to leak out from SR.[22] Additionally, an increased influx of calcium declined the ADP/O ratio and respiratory control, which increased the expression of mitochondria uncoupling proteins-3 (UCP-3) (~53%) and enhanced the generation of ROS/RNS.[23] Therefore, several factors are involved in the increased influx of Ca^{2+} into the intracellular space and mitochondrial matrix with two major factors, i.e., increased transmembrane permeability of Ca^{2+} and leakage of Ca^{2+} from oxidized RyR1 Ca^{2+} release channels of SR. Increased calcium influx to the mitochondria matrix and ROS activate the Ca^{2+}-dependent caspase and calpains to disrupt myofilaments and facilitate protein catabolism and apoptosis.[22]

Also, the unavailability of ATP triggers the activation of AMPK, which accelerates the dephosphorylation of FoxO3 in skeletal muscles.[2] Upregulated expression of FoxO3 was reinforced through the release of pro-inflammation cytokine/chemokines viz., interleukin-1beta (IL-1β), IL-6, tumor necrosis factor-alpha (TNF-α) infiltration, and translocation of nuclear factor kappa B (NF-κB) in SkM forming a positive feedback mechanism for the activation of 26S proteasomal subunits and promote proteolysis.[20] Nevertheless, some contradictions in the aforementioned statements need to be addressed carefully.

INCREASED RELEASE OF ROS AND SKELETAL MUSCLE ATROPHY

Elevated intracellular and mitochondrial matrix Ca^{2+} levels result in ROS/RNS formation above the normal levels due to the leakage of membranes, especially of mitochondria, which causes negative feedback mechanisms. ROS activates Jun N-terminal kinase (JNK1) and inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ) and protein kinase C-beta/delta (PKCβ/δ), which in turn inhibit the insulin receptor substrate-1 (IRS-1) complex **(Figure 1)** and cause insulin resistance in SkM.[24] In addition, JNK1 and IKKβ activate IκBα and NF-κB, which activate inducible nitric oxide synthase (iNOS) and generate NO to nitrosylates IRS-1, which has an additive effect on insulin resistance. Since SkM requires a lot of energy, it is widely recognized that insulin's downstream signaling pathways are crucial for maintaining central metabolism in all cell types, including SkM. Energy-deficient SkM switches toward protein catabolism to fulfill energy needs and generate more ROS/RNS.[25] ROS/RNS leads to lipid oxidation, protein modifications, and DNA damage, ultimately impairing the functionality of proteins and organelles and leading to SkM atrophy.[15] Further, increased ROS also upregulated the expression of myostatin, a member of the tumor growth factor-beta (TGF-β) family that mediated the protein degradation via TNF-α dependant NF-κB and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase signalling. Once, stimulated myostatin also triggers the activation of its downstream targets viz., MuRF-1 and Atrogin-1 in soleus SkM.[26] Also upregulated ROS, decreased the quality control of mitochondria to trigger the release of cytochrome C and reduce the oxidative capacity of mitochondria.[27] Clinical trials on patients of gastrointestinal cancer-induced cachexia showed the altered mitochondrial morphology associated with T tubulins of SkM due to increased expression of mitochondrial fission 1 (Fis1) ($p=0.03$) and increased inter myofibrillar mitochondrial area. This causes the increase in levels of p-p53 Ser46, caspases 8 Asp384/9, Asp315 significantly ($p = 0.037$) ($p = 0.046$) and reduces the mitofusion 1 (Mfn1) and Mfn2 of mitochondria.[28] However, their expression in denervated SkM is yet to be explored further. Similarly, the loss of contractile function in muscle due to nerve resection led to a reduced level of blood flow to promote a hypoxic state. Increased hypoxia contributes to the formation of ROS which in excess stimulates the release of a downstream target of muscle atrophy i.e. pro-inflammatory cytokine and chemokines to initiate the atrophy-related pathway of muscle proteolysis.[29] It has been reported that 14 days of denervation induce the release of TNF-α, IL-6, and IL-1β in the tibia anterior (TA) muscle to promote the Foxo3A expression in TA. Elevated Foxo3A levels increase muscle proteolysis through MuRF-1 and Atrogin-1 activation and autophagy-lysosomal system. Further, increased IL-6 led to the activation of its downstream signaling JAK2/STAT3 in TA muscles.[30]

In brief, ROS/RNS increases the rate of protein catabolism through ubiquitin-proteasome, calpain, caspases, apoptosis, mitophagy, and autophagy.[15] In contrast, another study observed that generated oxidative stress was not responsible for muscle loss after 3 days of nerve damage. In fact, on day 3, the activity of NADPH oxidase subunit Cybb was found to be upregulated, which directly enhances the translocation of NF-κB, while the amount of carbonylated and *malondialdehyde* (MDA) adducts remains low following 3 days of nerve damage.[31] Moreover, generated mitochondrial ROS (mROS) via PTP and MCU activity was enough to degenerate the NMJs degeneration but not sufficient to cause SkM atrophy.[27] Moreover, mROS promoted muscle growth via enhanced fiber branching in the quadriceps and gastrocnemius muscles.[32]

Increased levels of ROS are directly related to inflammation. ROS promotes the activation of IKKβ, which consequently activates NF-κB. NF-κB activation in skeletal muscle results in the breakdown of specific muscle proteins, the induction of

inflammatory and fibrotic responses, and the inhibition of muscle fiber regeneration following injury/atrophy.[15] NF-κB also induces the expression of MuRF-1, ubiquitin-proteasome units, as well as tissue degrading enzymes and inhibits the myogenic differentiation process, possibly via modulation of MyoD levels.[33] Therefore, it promotes SkM wasting in several ways.

Moreover, inhibition of IRS-1 by ROS as well as by inflammatory factors decreases the glucose uptake in SkM and promotes the activation of transcription factor FOXO.[34] Activation of FOXO increases the expression of atrogin-1 and E3 ligase to increase protein degradation via ubiquitin-proteasome; BNIP3, autophagy-related gene 12 (Atg12), and Atg8 to increase mitophagy and autophagy; and B-cell lymphoma 6 (Bcl-6), etc. to increase apoptosis in SkM.[15] Increased ROS after denervation impairs mitochondrial oxidative capacity by targeting Mfn1 and dynamin-related protein-1 (Drp1) to inhibit the mitochondrial respiration cycle. It also increases the expression of lysosomal protein for the initiation of mitophagy flux and reduces the expression of mitochondria housed PTEN-induced kinase 1 (Pink1), peroxiredoxin 2 (Prdx2), sirtuin 2/3/5 (Sirt2/3/5). A continuous decline in mitochondria proteins and antioxidant levels viz, superoxide dismutase (SOD), glutathione peroxides (GPx), etc led to activation of lysosomal mediated autophagy of muscle proteins after denervation.[35]

However, it was observed that excessive overload of ROS triggers the protein catabolism in SkM,[2] but some studies reported that ROS supports the cardiac hypertrophy in a TP53-inducible glycolysis and apoptosis regulator (TIGAR), potentially targets the p53-dependent manner, and inhibits apoptosis and autophagy.[36] Activated TIGAR was reported to modulate the glycolytic enzyme fructose-2,6-bisphosphate to regulate the intracellular oxidative stress and elevate the expression of NADPH for scavenging the GPx activity in a ROS-dependent manner[37] but its expression in denervation-induced SkM atrophy need to explore further. Similarly, an adequate amount of in-house ROS also regulates the IGF-1 pathway of muscle hypertrophy. It was observed that ROS facilitates the phosphorylation of tyrosine residue of the IRβ chain to activate Akt signaling of muscle hypertrophy. Also, low levels of hydrogen peroxides stimulated the ERK, Akt kinase, and p38 expression and escalated G-protein-dependent responses of α-adrenergic and angiotensin expression.[33, 34]

Conclusion

Overall, Ca^{2+} ions serve as the primary signaling molecules in muscles for the process of excitation-contraction coupling, as well as in the modulation of SkM plasticity. In addition to this crucial role, Ca^{2+} also plays a regulatory role in various processes, such as the facilitation of myosin-actin cross-bridging, protein synthesis, and protein degradation. However, under pathophysiological conditions, the Ca^{2+} leak channels elevate cytosolic Ca^{2+} levels, subsequently leading to the activation of calcium-dependent protease, namely calpains. Consequently, this activation of calpains leads to the degradation of myofilaments. Therefore, denervation-induced Ca^{2+} influx increases the generation of ROS, inflammation, and mitophagy-mediated catabolic pathways in SkM, which not only leads to reduced quality of life but also increases morbidity and mortality. Moreover, denervation activated the AMPK expression to inhibit the expression of SOD, GPx, and creatine kinase leading to the inhibition of Akt-mTOR signaling of muscle hypertrophy. Hence, ROS balance is critically responsible for myogenesis and muscle hypertrophy as well as for muscle proteolysis and is the prime marker of muscle atrophy. Therefore, further research is needed to unravel the intricate molecular pathways and interactions involved in muscle atrophy in different physiological contexts, paving the way for more effective therapeutic approaches.

Acknowledgment

Declared none.

Ethical Approval

Not applicable

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

References and Notes

1. B. Sharma, V. Dutt, N. Kaur, A. Mittal, R. Dabur. Tinospora cordifolia protects from skeletal muscle atrophy by alleviating oxidative stress and inflammation induced by sciatic denervation. *J Ethnopharmacol* **2020**, 254, 112720.
2. A. Yadav, S.S. Yadav, S. Singh, R. Dabur. Natural products: Potential therapeutic agents to prevent skeletal muscle atrophy. *Eur J Pharmacol* **2022**, 925, 174995.
3. S.R. Iyer, S.B. Shah, R.M. Lovering. The Neuromuscular Junction: Roles in Aging and Neuromuscular Disease. *Int J Mol Sci* **2021**, 22 (15), 8058.
4. A. Omar, K. Marwaha, P.C. Bollu. Physiology, Neuromuscular Junction. In *StatPearls*; StatPearls Publishing, Treasure Island (FL), **2024**.
5. S. Zou, B.-X. Pan. Post-synaptic specialization of the neuromuscular junction: junctional folds formation, function, and disorders. *Cell Biosci* **2022**, 12 (1), 93.
6. S. Jimsheleishvili, K. Marwaha, A.L. Sherman. Physiology, Neuromuscular Transmission. In *StatPearls*; StatPearls Publishing, Treasure Island (FL), **2024**.
7. J.A. Heiny, V.V. Kravtsova, F. Mandel, et al. The Nicotinic Acetylcholine Receptor and the Na,K-ATPase α2 Isoform Interact to Regulate Membrane Electrogenesis in Skeletal Muscle. *Journal of Biological Chemistry* **2010**, 285 (37), 28614–28626.
8. M. Kudlak, P. Tadi. Physiology, Muscarinic Receptor. In *StatPearls*; StatPearls Publishing, Treasure Island (FL), **2024**.
9. I.Y. Kuo, B.E. Ehrlich. Signaling in muscle contraction. *Cold Spring Harb Perspect Biol* **2015**, 7 (2), a006023.
10. W.A. Catterall. Sodium transport by the acetylcholine receptor of cultured muscle cells. *J. Biological Chem.* **1975**, 250 (5), 1776–1781.
11. S. Elenes, M. Decker, G.D. Cymes, C. Grosman. Decremental Response to High-Frequency Trains of Acetylcholine Pulses but Unaltered Fractional Ca2+ Currents in a Panel of “Slow-Channel Syndrome” Nicotinic Receptor Mutants. *J. General Physiology* **2009**, 133 (2), 151–169.
12. A. Vincent. Immunology of acetylcholine receptors in relation to myasthenia gravis. *Physiol Rev* **1980**, 60 (3), 756–824.
13. S. Gehlert, W. Bloch, F. Suhr. Ca2+-dependent regulations and signaling in skeletal muscle: from electro-mechanical coupling to adaptation. *Int J Mol Sci* **2015**, 16 (1), 1066–1095.
14. J. Zhou, K. Dhakal, J. Yi. Mitochondrial Ca(2+) uptake in skeletal muscle health and disease. *Sci China Life Sci* **2016**, 59 (8), 770–776.
15. A. Yadav, R. Dabur. Skeletal muscle atrophy after sciatic nerve damage: Mechanistic insights. *Eur. J. Pharmacology* **2024**, 970, 176506.
16. N. Garcia, M.M. Santafé, I. Salon, M.A. Lanuza, J. Tomàs. Expression of muscarinic acetylcholine receptors (M1-, M2-, M3- and M4-type) in

the neuromuscular junction of the newborn and adult rat. *Histol Histopathol* **2005**, 20 (3), 733–743.
17. J. Zhou. Ca2+-mediated coupling between neuromuscular junction and mitochondria in skeletal muscle. *Neurosci Lett* **2021**, 754, 135899.
18. M. Gleichmann, M.P. Mattson. Neuronal calcium homeostasis and dysregulation. *Antioxid Redox Signal* **2011**, 14 (7), 1261–1273.
19. A. De Mario, E. Trevellin, I. Piazza, et al. Mitochondrial Ca2+ signaling is a hallmark of specific adipose tissue-cancer crosstalk. *Sci Rep* **2024**, 14 (1), 8469.
20. A.Y. Baev, A.Y. Vinokurov, I.N. Novikova, et al. Interaction of Mitochondrial Calcium and ROS in Neurodegeneration. *Cells* **2022**, 11 (4), 706.
21. L. Modesti, A. Danese, V. Angela Maria Vitto, et al. Mitochondrial Ca2+ Signaling in Health, Disease and Therapy. *Cells* **2021**, 10 (6), 1317.
22. A.R. Marks. Targeting ryanodine receptors to treat human diseases. *J Clin Invest* **2023**, 133 (2), e162891.
23. A.J. Murray, M.A. Cole, C.A. Lygate, et al. Increased mitochondrial uncoupling proteins, respiratory uncoupling and decreased efficiency in the chronically infarcted rat heart. *J Mol Cell Cardiol* **2008**, 44 (4), 694–700.
24. R. Sartori, V. Romanello, M. Sandri. Mechanisms of muscle atrophy and hypertrophy: implications in health and disease. *Nat Commun* **2021**, 12 (1), 330.
25. S.S. Soskić, B.D. Dobutović, E.M. Sudar, et al. Regulation of Inducible Nitric Oxide Synthase (iNOS) and its Potential Role in Insulin Resistance, Diabetes and Heart Failure. *Open Cardiovasc Med J* **2011**, 5, 153–163.
26. M.-M. Chen, Y.-P. Zhao, Y. Zhao, S.-L. Deng, K. Yu. Regulation of Myostatin on the Growth and Development of Skeletal Muscle. *Front Cell Dev Biol* **2021**, 9, 785712.
27. D.B. Zorov, M. Juhaszova, S.J. Sollott. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev* **2014**, 94 (3), 909–950.
28. G.S. De Castro, E. Simoes, J.D.C.C. Lima, et al. Human Cachexia Induces Changes in Mitochondria, Autophagy and Apoptosis in the Skeletal Muscle. *Cancers* **2019**, 11 (9), 1264.
29. R.J. Rivers, C.J. Meininger. The Tissue Response to Hypoxia: How Therapeutic Carbon Dioxide Moves the Response toward Homeostasis and Away from Instability. *IJMS* **2023**, 24 (6), 5181.
30. C. Wu, L. Tang, X. Ni, et al. Salidroside Attenuates Denervation-Induced Skeletal Muscle Atrophy Through Negative Regulation of Pro-inflammatory Cytokine. *Front Physiol* **2019**, 10, 665.
31. Sofia Lourenço dos Santos. Role of oxidised protein repair for skeletal muscle homeostasis. **2016**.
32. B. Ahn, R. Ranjit, P. Premkumar, et al. Mitochondrial oxidative stress impairs contractile function but paradoxically increases muscle mass via fibre branching. *J cachexia sarcopenia muscle* **2019**, 10 (2), 411–428.
33. A. Thoma, A.P. Lightfoot. NF-kB and Inflammatory Cytokine Signalling: Role in Skeletal Muscle Atrophy. *Adv Exp Med Biol* **2018**, 1088, 267–279.
34. B. Ponugoti, G. Dong, D.T. Graves. Role of forkhead transcription factors in diabetes-induced oxidative stress. *Exp Diabetes Res* **2012**, 2012, 939751.
35. J.M. Memme, M. Slavin, N. Moradi, D.A. Hood. Mitochondrial Bioenergetics and Turnover during Chronic Muscle Disuse. *Int J Mol Sci* **2021**, 22 (10), 5179.
36. D. Shim, L. Duan, C.G. Maki. P53-regulated autophagy and its impact on drug resistance and cell fate. *Cancer Drug Resist* **2021**, 4 (1), 85–95.
37. I.I.C. Chio, D.A. Tuveson. ROS in Cancer: The Burning Question. *Trends Mol Med* **2017**, 23 (5), 411–429.
38. S. Sriram, S. Subramanian, D. Sathiakumar, et al. Modulation of reactive oxygen species in skeletal muscle by myostatin is mediated through NF-κB. *Aging Cell* **2011**, 10 (6), 931–948.
39. D. Zechner, D.J. Thuerauf, D.S. Hanford, P.M. McDonough, C.C. Glembotski. A role for the p38 mitogen-activated protein kinase pathway in myocardial cell growth, sarcomeric organization, and cardiac-specific gene expression. *J Cell Biol* **1997**, 139 (1), 115–127.