Muscle Delivery of Mitochondria-Targeted Drugs for Sarcopenia

Subjects: Gerontology

Contributor: Francesco Bellanti, Aurelio Lo Buglio, Gianluigi Vendemiale

An impairment in mitochondrial homeostasis plays a crucial role in the process of aging and contributes to the incidence of age-related diseases, including sarcopenia, which is defined as an age-dependent loss of muscle mass and strength. Mitochondrial dysfunction exerts a negative impact on several cellular activities, including bioenergetics, metabolism, and apoptosis. In sarcopenia, mitochondria homeostasis is disrupted because of reduced oxidative phosphorylation and ATP generation, the enhanced production of reactive species, and impaired antioxidant defense.

Keywords: sarcopenia ; mitochondrial dysfunction ; oxidative stress

1. Introduction

In recent years, life expectancy has enormously increased all over the world. This has been accompanied by growing health problems related to aging, since the extension of the expected lifespan is unavoidably followed by biological modifications that affect the human body. In particular, age-dependent changes related to muscle mass and function are notably evident as individuals become older and older. The loss of muscle mass during ageing, followed by a decline in physical function and mobility, is defined by the term "sarcopenia" [1]. According to a recently revised European consensus, sarcopenia is typically characterized by low muscle strength, quantity, and quality [2]. The pathogenesis of sarcopenia is extremely complicated and multifactorial, but most research suggests that modifications in mitochondrial biogenesis, morphology, function, and dynamics may represent the key process in disrupted muscle function and quality [[3]]. Progression in mitochondrial research and biomedical technology encourages the development of drugs specifically targeted to mitochondria for therapeutic use . Thus, researchers aim to stimulate the progression of muscle mitochondria-directed therapeutics for the management of sarcopenia.

2. Mitochondrial Involvement in Sarcopenia

2.1. Age-Related Alterations in Morphology and Dynamics of Skeletal Muscle Mitochondria

Research on morphology in aged skeletal muscle describes giant mitochondria with disrupted cristae [4]. Furthermore, compared with the skeletal muscle of young/adults, old SS mitochondria seem fragmented and positioned in a thin layer, while IMF mitochondria appear less reticular [5]. Of interest, a decrease in IMF size was reported in old people, particularly in women more than men, although there were no differences in skeletal muscle size between both sexes [6]. Morphological alterations in old skeletal muscle mitochondria may result from changes in mitochondrial dynamics, characterized by an imbalance that enhances fission rather than fusion [7]. Mitochondrial dynamics can be dysregulated by mtDNA mutations, since old mice expressing a defective mtDNA polymerase gamma exhibited enhanced mitochondrial fission in skeletal muscle [8]. Nevertheless, a higher mitochondrial fusion was described in skeletal muscle from old versus young mice [9]. A change toward mitochondrial fusion rather than fission was further described in the skeletal muscle of old hip-fractured patients [10]. A knock-out of fusion-related proteins (mitofusins, Mfn1/2) in skeletal muscle caused higher mtDNA mutations and tissue atrophy [11]. However, skeletal muscle degeneration and atrophy were described as a result of the deletion of the fission-related protein Drp1 [12]. Taken together, these studies suggest that modifications of mitochondrial dynamics in skeletal muscle and their commitment in sarcopenia need to be clarified.

2.2. Mitochondrial Dysfunction and Apoptosis in Old Skeletal Muscle

Dysfunctional mitochondria cause both the exhaustion of ATP and excess of reactive species, with the consequent initiation of damaging cellular pathways. In old skeletal muscle, decreases in mitochondrial activity of the enzymes involved in the tricarboxylic acid cycle, oxygen consumption, and ATP synthesis are reported [13]. Moreover, mitochondrial dysfunction triggers apoptosis, with a negative impact on skeletal muscle quality [14].

Among the worsened mitochondrial functions in old skeletal muscle, the activity of metabolic enzymes (such as citrate synthase) and oxidative phosphorylation (OXPHOS) complexes, protein synthesis, and ATP production rate (mostly caused by an increase in mitochondrial uncoupling) were described [15][16][17][18][19]. However, it is worth noting that mitochondrial function in old skeletal muscle can be preserved with durable and intense physical activity [20][21][22]. To comply with this statement, exercise-mimicking compounds, such as AMP-activated protein kinase or peroxisome proliferator-activated receptor-δ (PPAR-δ) agonists, might act synergistically with mitochondria-targeted therapies to improve muscle quality [23].

Age-dependent reduction in mitochondrial gene expression is described when the transcriptome of old skeletal muscle is compared to young people, although proteomic investigations are controversial, suggesting the need for further studies [24]. Notably, genes related to mitochondrial structure and function are downregulated in old women compared to men, suggesting that females may be more prone to age-dependent mitochondrial impairment in skeletal muscle [25].

In sarcopenia, mtDNA and mitochondrial electron transport chain (ETC) changes are triggered by oxidative stress [26]. Indeed, the highest prevalence of mtDNA deletions is reported in those skeletal muscle fibers exposed to oxidative injury [27][28]. Increased mtDNA deletions are related to modifications of mitochondrial enzymes in old primates and humans [29][30]. An inactive lifestyle in old age is related to mitochondrial dysfunction and oxidative injury in human skeletal muscle, so physical activity may prevent mitochondrial-dependent sarcopenia [30][31]. Induced mtDNA mutations in the skeletal muscle of mice caused a disruption in ETC assembly and function, impairing mitochondrial bioenergetics and ATP homeostasis, and triggering apoptosis and sarcopenia [32]. Dysfunctional mitochondria were also reported in spinal motor neurons from old humans, contributing to the denervation and collapse of skeletal muscle quality [33]. Notably, the denervation of skeletal muscle fibers triggers mitochondrial reactive species even in nearby innervated fibers, indicating a collateral mechanism in sarcopenia [34].

Dysfunctional mitochondria may trigger apoptosis in old skeletal muscle. Indeed, mitochondria from aged skeletal muscle exhibit a high production rate of reactive species and low calcium internalization, with the consequent opening of the mitochondrial permeability transition pore (mPTP), the release of cytochrome c, and DNA fragmentation, all markers of apoptosis [19][35]. Training exercises may reduce the mitochondrial release of proapoptotic proteins and the resultant DNA fragmentation [36][37]. Mitochondrial dysfunction may also induce a caspase-independent apoptotic pathway that contributes to the disruption of muscle quality in aging [38]. The calcium retention capacity was shown to be reduced in skeletal muscle mitochondria from old men, indicating mPTP sensitization to apoptosis [35]. Thus, mitochondria-dependent apoptosis in skeletal muscle represents a potential therapeutic target to counterbalance sarcopenia, as suggested by both in vitro and ex vivo studies [39][40].

2.3. Age-Dependent Alterations in Skeletal Muscle Mitochondria Biogenesis and Mitophagy

Alterations in skeletal muscle quality are also dependent on changes in mitochondrial biogenesis. Mitochondrial homeostasis in skeletal muscle is under the control of the peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α, the master regulator of mitochondrial biogenesis, which is promoted by contractile activity and induces the switching from glycolytic toward oxidative fibers [41]. Nevertheless, an age-dependent decrease in mitochondrial biogenesis may be sustained by the defective response of PGC-1α to exercise [42]. The decreased mitochondrial content in old skeletal muscle may also be dependent on lower PGC-1α expression, which has been described both in slow- and in fast-twitch fibers [4][7][16]. Nevertheless, other studies have described opposite results related to the expression level of the mitochondrial transcription factor A (Tfam), a downstream main PGC-1α transcription factor, in old skeletal muscle [43][44][45].

The limited capacity of senescent skeletal muscle cells to remove injured mitochondria (mitophagy) could be a further cause of mitochondrial alteration. Nevertheless, studies on skeletal muscle from rodents show debated results on mitophagy modulators [46][47][48]. PGC-1α overexpression in skeletal muscle inhibits mitophagy, which appears enhanced in aging [46]. Genes related to mitophagy were described as downregulated in a cross-sectional study on physically inactive frail old women [49]. On the contrary, mitophagy and its regulatory proteins were increased in rodent models of sarcopenia [50][51]. Another study indicated that lysosomal dysfunction may cause an accumulation of disrupted mitochondria in the skeletal muscle of old mice [52].

3. Muscle Mitochondria-Targeted Therapy for the Management of Sarcopenia

3.1. Mitochondria-Targeted Delivery Systems

Mitochondrial delivery strategies can be classified either as referring to the molecular size and type or considering the molecular mechanism [53][54]. According to the first, the best strategy to target mitochondria for the treatment of sarcopenia consists of the use of 1–1000 nm sized particles, which can directly trigger myotubes or inflammatory cells [55][56][57]. According to the latter, passive and active mechanisms are described. Passive targeting relies on the physical and chemical properties of carrier systems, while active targeting refers to specific interactions (ligand–receptor or antigen–antibody) at mitochondrial sites [58].

3.1.1. Passive Delivery

Several small-sized compounds can be highly localized within mitochondria because of their biochemical and biophysical features (lipophilicity and/or positive charge). Classified as delocalized lipophilic cations (DLCs), these compounds easily cross mitochondrial membranes and locate in the matrix. DLCs include tetraphenylphosphonium (TPP^+) or its methylated form ($TPMP^+$), dequalinium (DQA), and guanidine [58]. DLCs are conjugated to deliver antioxidant compounds, to selectively transport DNA or anticancer agents, sorbitol, metals, and copolymers [58]. Even though DLCs allow for the mitochondrial administration of a specific drug dose, preventing toxicity and resistance, their delivery is limited to electrically neutral and very small conjugates, together with an increased risk of depolarization [59]. Szeto–Schiller (SS) peptides are cell-permeable short peptides (less than 10 amino acids) with antioxidant properties, whose cellular uptake is only dependent on concentration, but not on an electric charge, preventing the risk of depolarization [60]. Liposomes are spherical compounds consisting of phosphatidylglycerol, phosphatidylcholine, and cholesterol, with a hydrophilic core surrounded by a lipid bilayer [61]. Liposomes are nontoxic and can deliver large-sized drugs, including antioxidants, mitochondria-targeted molecules, or even mtDNA [62][63].

3.1.2. Active Delivery

A different strategy to deliver compounds within mitochondria consists of the use of peptides, which are specifically recognized by signal sequences and cleaved off after effective import.

Cell-penetrating peptides (CPPs), such as R8 (RRRRRRRR) and TAT (RKKRRQRRR), are used to enhance the delivery of oligonucleotides, peptides, proteins, and liposomes [64][65].

Mitochondria signal peptides (MSPs) or mitochondria-targeting sequences (MTSs) are normally used to import proteins synthetized in ribosomes within mitochondria [66]. These MSPs or MTSs can be conjugated to nonmitochondrial compounds to form chimeric molecules that are specifically recognized by mitochondrial import machinery, selectively delivering to the intermembrane space, inner membrane, or matrix [66].

Mitochondria-penetrating peptides (MPPs) are artificial compounds based on a CPP strategy, but enriched with positively charged peptides and extra lipophilic amino acids that can efficiently cross the mitochondrial bilayer and interact with the inner mitochondrial membrane [67]. Indeed, compounds covalently conjugated to MMPs are able to mostly accumulate in mitochondria rather than the cytoplasm or nucleus [68].

3.2. Mitochondria-Targeted Therapy in Muscle Tissue

To date, exercise is the sole proven therapy for sarcopenia, since it can limit modifications induced by muscle aging [69][70][71]. Nevertheless, several sarcopenic patients are not able to exercise because of clinical complications and/or protracted immobilization. Consequently, the development of compounds that limit the loss of skeletal muscle mass and function is strongly encouraged. To be significantly effective, these compounds should be conveyed using a suitable drug delivery system. A main determinant strategy for developing such compounds is muscle-targeting delivery systems. Among examples of muscle-targeting peptides, the heptapeptide sequence ASSLNIA improves the specificity for binding to skeletal muscle by screening a random phage display library [72]. The 5-polyamidoamine dendrimer (G5-PAMAM) modified with ASSLNIA may synergistically improve skeletal muscle gene delivery [73]. The 12-mer peptide M12, which increases the binding affinity to myoblasts, conjugated with phosphorodiamidate morpholino oligomers may improve muscle function [74].

A promising approach to boost mitochondrial function in muscles consists of increasing intracellular NAD^+ by inhibiting enzymes that deplete its intracellular levels. The prolonged utilization of MRL-45696, a dual inhibitor of poly(ADP-ribose) polymerases 1 and 2 (PARP1 and PARP2, which consume NAD^+), improves mitochondrial function in mouse skeletal muscle [75]. The nicotinic acid derivative acipimox, an NAD^+ precursor, is able to directly enhance skeletal muscle mitochondrial function in humans [76].

Evidence on the efficacy of mitochondria-targeted drug delivery in skeletal muscle was provided in several preclinical studies. Mitoquinone Q, a mitochondria-targeted antioxidant, was able to improve muscle strength and mass in a murine model of cancer cachexia, stimulating beta-oxidation and promoting a shift from glycolytic to oxidative metabolism in muscle fibers [77]. Mito-TEMPOL, a mitochondria-targeted superoxide dismutase mimetic, prevents muscle weakness and wasting via the improvement in mitochondrial function in models of sepsis and uremia [78][79]. The mitochondria-targeted Szeto–Schiller peptide SS-31 was shown to improve exercise tolerance by increasing mitochondrial quality without mitochondrial content in aged mice [80].

4. Conclusions

Innovative pharmacology is able to produce molecules that can modulate mitochondria in several ways. However, these molecules need to be tested in vivo for the treatment of sarcopenia. Preclinical experiments strongly advise for their potential efficacy in preserving mitochondrial quality and function, counterbalancing oxidative stress and preventing mitochondrial apoptosis. The development of molecules targeted to skeletal muscle mitochondria could overwhelm several challenges associated with actual therapies, increasing the efficacy and decreasing toxicity. Even though mitochondrial medicine is developing, current applications in the treatment of sarcopenia support future clinical studies.

References

1. Rosenberg, I.H. Sarcopenia: Origins and clinical relevance. J. Nutr. 1997, 127, 990S–991S.
2. Cruz-Jentoft, A.J.; Bahat, G.; Bauer, J.; Boirie, Y.; Bruyere, O.; Cederholm, T.; Cooper, C.; Landi, F.; Rolland, Y.; Sayer, A.A.; et al. Sarcopenia: Revised European consensus on definition and diagnosis. Age Ageing 2019, 48, 601.
3. Bellanti, F.; Lo Buglio, A.; Vendemiale, G; Mitochondrial Impairment in Sarcopenia. *Biology* **2021**, *10*, 31, 10.3390/biology10010031.
4. Beregi, E.; Regius, O.; Huttl, T.; Gobl, Z. Age-related changes in the skeletal muscle cells. Z. Gerontol. 1988, 21, 83–86.
5. Iqbal, S.; Ostojic, O.; Singh, K.; Joseph, A.M.; Hood, D.A. Expression of mitochondrial fission and fusion regulatory proteins in skeletal muscle during chronic use and disuse. Muscle Nerve 2013, 48, 963–970.
6. Callahan, D.M.; Toth, M.J. Skeletal muscle protein metabolism in human heart failure. Curr. Opin. Clin. Nutr. Metab. Care 2013, 16, 66–71.
7. Joseph, A.M.; Adhihetty, P.J.; Buford, T.W.; Wohlgemuth, S.E.; Lees, H.A.; Nguyen, L.M.; Aranda, J.M.; Sandesara, B.D.; Pahor, M.; Manini, T.M.; et al. The impact of aging on mitochondrial function and biogenesis pathways in skeletal muscle of sedentary high- and low-functioning elderly individuals. Aging Cell 2012, 11, 801–809.
8. Joseph, A.M.; Adhihetty, P.J.; Wawrzyniak, N.R.; Wohlgemuth, S.E.; Picca, A.; Kujoth, G.C.; Prolla, T.A.; Leeuwenburgh, C. Dysregulation of mitochondrial quality control processes contribute to sarcopenia in a mouse model of premature aging. PLoS ONE 2013, 8, e69327.
9. Leduc-Gaudet, J.P.; Auger, M.J.; St Jean, P.F.; Gouspillou, G. Towards a better understanding of the role played by mitochondrial dynamics and morphology in skeletal muscle atrophy. J. Physiol. 2015, 593, 2993–2994.
10. Picca, A.; Calvani, R.; Lorenzi, M.; Menghi, A.; Galli, M.; Vitiello, R.; Randisi, F.; Bernabei, R.; Landi, F.; Marzetti, E. Mitochondrial dynamics signaling is shifted toward fusion in muscles of very old hip-fractured patients: Results from the Sarcopenia in HIp FracTure (SHIFT) exploratory study. Exp. Gerontol. 2017, 96, 63–67.
11. Correia-Melo, C.; Ichim, G.; Tait, S.W.; Passos, J.F. Depletion of mitochondria in mammalian cells through enforced mitophagy. Nat. Protoc. 2017, 12, 183–194.
12. Favaro, G.; Romanello, V.; Varanita, T.; Andrea, D.M.; Morbidoni, V.; Tezze, C.; Albiero, M.; Canato, M.; Gherardi, G.; De, S.D.; et al. DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. Nat. Commun. 2019, 10, 2576.
13. Marzetti, E.; Calvani, R.; Cesari, M.; Buford, T.W.; Lorenzi, M.; Behnke, B.J.; Leeuwenburgh, C. Mitochondrial dysfunction and sarcopenia of aging: From signaling pathways to clinical trials. Int. J. Biochem. Cell Biol. 2013, 45, 2288–2301.

14. Marzetti, E.; Leeuwenburgh, C. Skeletal muscle apoptosis, sarcopenia and frailty at old age. Exp. Gerontol. 2006, 41, 1234–1238.

15. Boffoli, D.; Scacco, S.C.; Vergari, R.; Solarino, G.; Santacroce, G.; Papa, S. Decline with age of the respiratory chain activity in human skeletal muscle. Biochim. Biophys. Acta 1994, 1226, 73–82.

16. Rooyackers, O.E.; Adey, D.B.; Ades, P.A.; Nair, K.S. Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. Proc. Natl. Acad. Sci. USA 1996, 93, 15364–15369.

17. Short, K.R.; Bigelow, M.L.; Kahl, J.; Singh, R.; Coenen-Schimke, J.; Raghavakaimal, S.; Nair, K.S. Decline in skeletal muscle mitochondrial function with aging in humans. Proc. Natl. Acad. Sci. USA 2005, 102, 5618–5623.

18. Marcinek, D.J.; Schenkman, K.A.; Ciesielski, W.A.; Lee, D.; Conley, K.E. Reduced mitochondrial coupling in vivo alters cellular energetics in aged mouse skeletal muscle. J. Physiol. 2005, 569, 467–473.

19. Chabi, B.; Ljubicic, V.; Menzies, K.J.; Huang, J.H.; Saleem, A.; Hood, D.A. Mitochondrial function and apoptotic susceptibility in aging skeletal muscle. Aging Cell 2008, 7, 2–12.

20. Kent-Braun, J.A.; Ng, A.V. Skeletal muscle oxidative capacity in young and older women and men. J. Appl. Physiol. 2000, 89, 1072–1078.

21. Picard, M.; Ritchie, D.; Thomas, M.M.; Wright, K.J.; Hepple, R.T. Alterations in intrinsic mitochondrial function with aging are fiber type-specific and do not explain differential atrophy between muscles. Aging Cell 2011, 10, 1047–1055.

22. Johnson, M.L.; Robinson, M.M.; Nair, K.S. Skeletal muscle aging and the mitochondrion. Trends Endocrinol. Metab. 2013, 24, 247–256.

23. Cento, A.S.; Leigheb, M.; Caretti, G.; Penna, F. Exercise and Exercise Mimetics for the Treatment of Musculoskeletal Disorders. Curr. Osteoporos. Rep. 2022, 20, 249–259.

24. Carter, H.N.; Chen, C.C.; Hood, D.A. Mitochondria, muscle health, and exercise with advancing age. Physiology 2015, 30, 208–223.

25. Liu, D.; Sartor, M.A.; Nader, G.A.; Pistilli, E.E.; Tanton, L.; Lilly, C.; Gutmann, L.; IglayReger, H.B.; Visich, P.S.; Hoffman, E.P.; et al. Microarray analysis reveals novel features of the muscle aging process in men and women. J. Gerontol. A Biol. Sci. Med. Sci. 2013, 68, 1035–1044.

26. Bua, E.A.; McKiernan, S.H.; Wanagat, J.; McKenzie, D.; Aiken, J.M. Mitochondrial abnormalities are more frequent in muscles undergoing sarcopenia. J. Appl. Physiol. 2002, 92, 2617–2624.

27. Aiken, J.; Bua, E.; Cao, Z.; Lopez, M.; Wanagat, J.; McKenzie, D.; McKiernan, S. Mitochondrial DNA deletion mutations and sarcopenia. Ann. NY Acad. Sci. 2002, 959, 412–423.

28. McKenzie, D.; Bua, E.; McKiernan, S.; Cao, Z.; Aiken, J.M. Mitochondrial DNA deletion mutations: A causal role in sarcopenia. Eur. J. Biochem. 2002, 269, 2010–2015.

29. McKiernan, S.H.; Colman, R.; Lopez, M.; Beasley, T.M.; Weindruch, R.; Aiken, J.M. Longitudinal analysis of early stage sarcopenia in aging rhesus monkeys. Exp. Gerontol. 2009, 44, 170–176.

30. Safdar, A.; Hamadeh, M.J.; Kaczor, J.J.; Raha, S.; deBeer, J.; Tarnopolsky, M.A. Aberrant mitochondrial homeostasis in the skeletal muscle of sedentary older adults. PLoS ONE 2010, 5, e10778.

31. Dodds, R.M.; Davies, K.; Granic, A.; Hollingsworth, K.G.; Warren, C.; Gorman, G.; Turnbull, D.M.; Sayer, A.A. Mitochondrial respiratory chain function and content are preserved in the skeletal muscle of active very old men and women. Exp. Gerontol. 2018, 113, 80–85.

32. Hiona, A.; Sanz, A.; Kujoth, G.C.; Pamplona, R.; Seo, A.Y.; Hofer, T.; Someya, S.; Miyakawa, T.; Nakayama, C.; Samhan-Arias, A.K.; et al. Mitochondrial DNA mutations induce mitochondrial dysfunction, apoptosis and sarcopenia in skeletal muscle of mitochondrial DNA mutator mice. PLoS ONE 2010, 5, e11468.

33. Rygiel, K.A.; Grady, J.P.; Turnbull, D.M. Respiratory chain deficiency in aged spinal motor neurons. Neurobiol. Aging 2014, 35, 2230–2238.

34. Pollock, N.; Staunton, C.A.; Vasilaki, A.; McArdle, A.; Jackson, M.J. Denervated muscle fibers induce mitochondrial peroxide generation in neighboring innervated fibers: Role in muscle aging. Free Radic. Biol. Med. 2017, 112, 84–92.

35. Gouspillou, G.; Sgarioto, N.; Kapchinsky, S.; Purves-Smith, F.; Norris, B.; Pion, C.H.; Barbat-Artigas, S.; Lemieux, F.; Taivassalo, T.; Morais, J.A.; et al. Increased sensitivity to mitochondrial permeability transition and myonuclear translocation of endonuclease G in atrophied muscle of physically active older humans. FASEB J. 2014, 28, 1621–1633.

36. Song, W.; Kwak, H.B.; Lawler, J.M. Exercise training attenuates age-induced changes in apoptotic signaling in rat skeletal muscle. Antioxid. Redox Signal. 2006, 8, 517–528.

37. Adhihetty, P.J.; Taivassalo, T.; Haller, R.G.; Walkinshaw, D.R.; Hood, D.A. The effect of training on the expression of mitochondrial biogenesis- and apoptosis-related proteins in skeletal muscle of patients with mtDNA defects. Am. J. Physiol. Endocrinol. Metab. 2007, 293, E672–E680.

38. Otera, H.; Mihara, K. Molecular mechanisms and physiologic functions of mitochondrial dynamics. J. Biochem. 2011, 149, 241–251.

39. Salucci, S.; Battistelli, M.; Baldassarri, V.; Burini, D.; Falcieri, E.; Burattini, S. Melatonin prevents mitochondrial dysfunctions and death in differentiated skeletal muscle cells. Microsc. Res. Tech. 2017, 80, 1174–1181.

40. Sayed, R.K.A.; Fernandez-Ortiz, M.; Diaz-Casado, M.E.; Rusanova, I.; Rahim, I.; Escames, G.; Lopez, L.C.; Mokhtar, D.M.; Acuna-Castroviejo, D. The Protective Effect of Melatonin against Age-Associated, Sarcopenia-Dependent Tubular Aggregate Formation, Lactate Depletion, and Mitochondrial Changes. J. Gerontol. A Biol. Sci. Med. Sci. 2018, 73, 1330–1338.

41. Akimoto, T.; Pohnert, S.C.; Li, P.; Zhang, M.; Gumbs, C.; Rosenberg, P.B.; Williams, R.S.; Yan, Z. Exercise stimulates Pgc-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway. J. Biol. Chem. 2005, 280, 19587–19593.

42. Derbre, F.; Gomez-Cabrera, M.C.; Nascimento, A.L.; Sanchis-Gomar, F.; Martinez-Bello, V.E.; Tresguerres, J.A.; Fuentes, T.; Gratas-Delamarche, A.; Monsalve, M.; Vina, J. Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1alpha to exercise training. Age 2012, 34, 669–679.

43. Lezza, A.M.; Pesce, V.; Cormio, A.; Fracasso, F.; Vecchiet, J.; Felzani, G.; Cantatore, P.; Gadaleta, M.N. Increased expression of mitochondrial transcription factor A and nuclear respiratory factor-1 in skeletal muscle from aged human subjects. FEBS Lett. 2001, 501, 74–78.

44. Masuyama, M.; Iida, R.; Takatsuka, H.; Yasuda, T.; Matsuki, T. Quantitative change in mitochondrial DNA content in various mouse tissues during aging. Biochim. Biophys. Acta 2005, 1723, 302–308.

45. Pesce, V.; Cormio, A.; Fracasso, F.; Lezza, A.M.; Cantatore, P.; Gadaleta, M.N. Age-related changes of mitochondrial DNA content and mitochondrial genotypic and phenotypic alterations in rat hind-limb skeletal muscles. J. Gerontol. A Biol. Sci. Med. Sci. 2005, 60, 715–723.

46. Yeo, D.; Kang, C.; Gomez-Cabrera, M.C.; Vina, J.; Ji, L.L. Intensified mitophagy in skeletal muscle with aging is downregulated by PGC-1alpha overexpression in vivo. Free Radic. Biol. Med. 2019, 130, 361–368.

47. Herbst, A.; Lee, C.C.; Vandiver, A.R.; Aiken, J.M.; McKenzie, D.; Hoang, A.; Allison, D.; Liu, N.; Wanagat, J. Mitochondrial DNA deletion mutations increase exponentially with age in human skeletal muscle. Aging Clin. Exp. Res. 2021, 33, 1811–1820.

48. Kim, C.; Hwang, J.K. The 5,7-Dimethoxyflavone Suppresses Sarcopenia by Regulating Protein Turnover and Mitochondria Biogenesis-Related Pathways. Nutrients 2020, 12, 1079.

49. Drummond, M.J.; Addison, O.; Brunker, L.; Hopkins, P.N.; McClain, D.A.; Lastayo, P.C.; Marcus, R.L. Downregulation of E3 ubiquitin ligases and mitophagy-related genes in skeletal muscle of physically inactive, frail older women: A cross-sectional comparison. J. Gerontol. A Biol. Sci. Med. Sci. 2014, 69, 1040–1048.

50. Chen, C.C.W.; Erlich, A.T.; Crilly, M.J.; Hood, D.A. Parkin is required for exercise-induced mitophagy in muscle: Impact of aging. Am. J. Physiol. Endocrinol. Metab. 2018, 315, E404–E415.

51. Carter, H.N.; Kim, Y.; Erlich, A.T.; Zarrin-Khat, D.; Hood, D.A. Autophagy and mitophagy flux in young and aged skeletal muscle following chronic contractile activity. J. Physiol. 2018, 596, 3567–3584.

52. O’Leary, M.F.; Vainshtein, A.; Iqbal, S.; Ostojic, O.; Hood, D.A. Adaptive plasticity of autophagic proteins to denervation in aging skeletal muscle. Am. J. Physiol. Cell Physiol. 2013, 304, C422–C430.

53. Yamada, Y.; Harashima, H. Mitochondrial drug delivery systems for macromolecule and their therapeutic application to mitochondrial diseases. Adv. Drug Deliv. Rev. 2008, 60, 1439–1462.

54. Fulda, S.; Galluzzi, L.; Kroemer, G. Targeting mitochondria for cancer therapy. Nat. Rev. Drug Discov. 2010, 9, 447–464.

55. Raimondo, T.M.; Mooney, D.J. Functional muscle recovery with nanoparticle-directed M2 macrophage polarization in mice. Proc. Natl. Acad. Sci. USA 2018, 115, 10648–10653.

56. Guglielmi, V.; Carton, F.; Vattemi, G.; Arpicco, S.; Stella, B.; Berlier, G.; Marengo, A.; Boschi, F.; Malatesta, M. Uptake and intracellular distribution of different types of nanoparticles in primary human myoblasts and myotubes. Int. J. Pharm. 2019, 560, 347–356.

57. Maretti, E.; Molinari, S.; Battini, R.; Rustichelli, C.; Truzzi, E.; Iannuccelli, V.; Leo, E. Design, Characterization, and In Vitro Assays on Muscle Cells of Endocannabinoid-like Molecule Loaded Lipid Nanoparticles for a Therapeutic Anti-

Inflammatory Approach to Sarcopenia. Pharmaceutics 2022, 14, 648.

58. Serviddio, G.; Bellanti, F.; Sastre, J.; Vendemiale, G.; Altomare, E. Targeting mitochondria: A new promising approach for the treatment of liver diseases. Curr. Med. Chem. 2010, 17, 2325–2337.

59. Armstrong, J.S. Mitochondrial medicine: Pharmacological targeting of mitochondria in disease. Br. J. Pharmacol. 2007, 151, 1154–1165.

60. Szeto, H.H. Cell-permeable, mitochondrial-targeted, peptide antioxidants. AAPS J. 2006, 8, E277–E283.

61. Akbarzadeh, A.; Rezaei-Sadabady, R.; Davaran, S.; Joo, S.W.; Zarghami, N.; Hanifehpour, Y.; Samiei, M.; Kouhi, M.; Nejati-Koshki, K. Liposome: Classification, preparation, and applications. Nanoscale Res. Lett. 2013, 8, 102.

62. Yamada, Y.; Harashima, H. Delivery of bioactive molecules to the mitochondrial genome using a membrane-fusing, liposome-based carrier, DF-MITO-Porter. Biomaterials 2012, 33, 1589–1595.

63. Wongrakpanich, A.; Geary, S.M.; Joiner, M.L.; Anderson, M.E.; Salem, A.K. Mitochondria-targeting particles. Nanomedicine 2014, 9, 2531–2543.

64. Torchilin, V.P.; Rammohan, R.; Weissig, V.; Levchenko, T.S. TAT peptide on the surface of liposomes affords their efficient intracellular delivery even at low temperature and in the presence of metabolic inhibitors. Proc. Natl. Acad. Sci. USA 2001, 98, 8786–8791.

65. Joliot, A.; Prochiantz, A. Transduction peptides: From technology to physiology. Nat. Cell Biol. 2004, 6, 189–196.

66. Lu, P.; Bruno, B.J.; Rabenau, M.; Lim, C.S. Delivery of drugs and macromolecules to the mitochondria for cancer therapy. J. Control. Release 2016, 240, 38–51.

67. Horton, K.L.; Stewart, K.M.; Fonseca, S.B.; Guo, Q.; Kelley, S.O. Mitochondria-penetrating peptides. Chem. Biol. 2008, 15, 375–382.

68. Chamberlain, G.R.; Tulumello, D.V.; Kelley, S.O. Targeted delivery of doxorubicin to mitochondria. ACS Chem. Biol. 2013, 8, 1389–1395.

69. Landi, F.; Marzetti, E.; Martone, A.M.; Bernabei, R.; Onder, G. Exercise as a remedy for sarcopenia. Curr. Opin. Clin. Nutr. Metab. Care 2014, 17, 25–31.

70. Phu, S.; Boersma, D.; Duque, G. Exercise and Sarcopenia. J. Clin. Densitom. 2015, 18, 488–492.

71. Yoo, S.Z.; No, M.H.; Heo, J.W.; Park, D.H.; Kang, J.H.; Kim, S.H.; Kwak, H.B. Role of exercise in age-related sarcopenia. J. Exerc. Rehabil. 2018, 14, 551–558.

72. Samoylova, T.I.; Smith, B.F. Elucidation of muscle-binding peptides by phage display screening. Muscle Nerve 1999, 22, 460–466.

73. Jativa, S.D.; Thapar, N.; Broyles, D.; Dikici, E.; Daftarian, P.; Jimenez, J.J.; Daunert, S.; Deo, S.K. Enhanced Delivery of Plasmid DNA to Skeletal Muscle Cells using a DLC8-Binding Peptide and ASSLNIA-Modified PAMAM Dendrimer. Mol. Pharm. 2019, 16, 2376–2384.

74. Gao, X.; Zhao, J.; Han, G.; Zhang, Y.; Dong, X.; Cao, L.; Wang, Q.; Moulton, H.M.; Yin, H. Effective dystrophin restoration by a novel muscle-homing peptide-morpholino conjugate in dystrophin-deficient mdx mice. Mol. Ther. 2014, 22, 1333–1341.

75. Pirinen, E.; Canto, C.; Jo, Y.S.; Morato, L.; Zhang, H.; Menzies, K.J.; Williams, E.G.; Mouchiroud, L.; Moullan, N.; Hagberg, C.; et al. Pharmacological Inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle. Cell Metab. 2014, 19, 1034–1041.

76. van de Weijer, T.; Phielix, E.; Bilet, L.; Williams, E.G.; Ropelle, E.R.; Bierwagen, A.; Livingstone, R.; Nowotny, P.; Sparks, L.M.; Paglialunga, S.; et al. Evidence for a direct effect of the NAD+ precursor acipimox on muscle mitochondrial function in humans. Diabetes 2015, 64, 1193–1201.

77. Pin, F.; Huot, J.R.; Bonetto, A. The Mitochondria-Targeting Agent MitoQ Improves Muscle Atrophy, Weakness and Oxidative Metabolism in C26 Tumor-Bearing Mice. Front. Cell Dev. Biol. 2022, 10, 861622.

78. Supinski, G.S.; Wang, L.; Schroder, E.A.; Callahan, L.A.P. MitoTEMPOL, a mitochondrial targeted antioxidant, prevents sepsis-induced diaphragm dysfunction. Am. J. Physiol. Lung Cell Mol. Physiol. 2020, 319, L228–L238.

79. Liu, Y.; Perumal, E.; Bi, X.; Wang, Y.; Ding, W. Potential mechanisms of uremic muscle wasting and the protective role of the mitochondria-targeted antioxidant Mito-TEMPO. Int. Urol. Nephrol. 2020, 52, 1551–1561.

80. Campbell, M.D.; Duan, J.; Samuelson, A.T.; Gaffrey, M.J.; Merrihew, G.E.; Egertson, J.D.; Wang, L.; Bammler, T.K.; Moore, R.J.; White, C.C.; et al. Improving mitochondrial function with SS-31 reverses age-related redox stress and improves exercise tolerance in aged mice. Free Radic. Biol. Med. 2019, 134, 268–281.