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Cellular mechanism of immobilization-induced muscle atrophy: A mini review

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ABSTRACT

It is well-established that regular contraction maintains morphological and functional integrity of skeletal muscle, whereas rigorous exercise training can upregulate muscle metabolic and contractile function. However, when muscles stop contraction, such as during immobilization (IM) and denervation, withdrawal of IGF/Akt/mTOR signaling allows FoxO-controlled protein degradation pathways to dominate. Mitochondria play an important role in regulating both protein synthesis and degradation via several redox sensitive signaling pathways such as mitochondrial biogenesis, fusion and fission dynamics, ubiquitin-proteolysis, autophagy/mitophagy, and apoptosis. During prolonged IM, downregulation of PGC-1α and increased mitochondrial oxidative damage facilitate fission protein and inflammatory cytokine production and activate mitophagic process, leading to a vicious cycle of protein degradation. This "mitostasis theory of muscle atrophy" is the opposite pathway of hormesis, which defines enhanced muscle function with contractile overload. The demonstration that PGC-1α overexpression via transgene or in vivo DNA transfection can successfully restore mitochondrial homeostasis and reverse myocyte atrophy supports such a proposition. Understanding the mechanism governing mitostasis can be instrumental to the treatment of muscle atrophy associated with bedrest, cancer cachexia and sarcopenia.

Introduction

The concept of hormesis may be applied to skeletal muscle adaptation and remodeling in response to increased contractile activity as a stress.[1,2] Although exercise itself is not a specific hormetic stimulus, numerous biochemical and physiological changes take place during myocyte contraction that elicit hormetic effects.[3] Among the best-known hormetic effects studied to date include upregulation of antioxidant network, mitochondrial biogenesis, cardiac protection against ischemia-reperfusion, heat tolerance, adaptation to hypoglycemia, and muscle hypertrophy.[4]

In contrast, reduction, restriction or complete cessation of contractile activity due to denervation, bed rest, microgravity and senescence can lead to loss of muscle mass and function.[5] Numerous studies suggest that muscle immobilization (IM) enhances proteolysis, oxidative stress, inflammation, and metabolic disturbance.[6,7] Understanding the cellular mechanisms by which IM induces muscle atrophy and functional deterioration will not only elucidate the signaling pathways controlling muscle protein synthesis and degradation, but will also provide insights into development of therapeutic treatment for patients suffering from muscle wasting.

Muscle immobilization-a dehormetic process

Two parallel events occur during muscle IM: protein synthesis is decreased due to the diminished stimulation from the IGF-Akt-mTOR axis, whereas protein degradation is enhanced, triggered by activation of the ubiquitin-proteolysis pathway and the autophagy-lysosomal pathway, controlled mainly by the FoxO subfamily transcription factors.[7] Two endpoint enzymes responsible for the activation of protein degradation are the muscle-specific E3 ubiquitin ligases Atrogin-1 and MuRF1.[8,9] Atrogin-1 and MuRF1 control the ubiquitination and degradation of both regulatory (e.g. calcineurin and MyoD) and structural (e.g. myosin and troponin I) proteins.[10–13] Notably, remobilization (RM) of a muscle previously immobilized for an extended period of time does not quickly reverse IM effects, rather, IM-RM has been shown to activate NFκB pathway and subsequently stimulate the expression of pro-inflammatory cytokines such as TNFα, IL-1β, IL-6 and inflammatory myokines, and oxidative stress.[14]

FoxO signaling plays a key role in skeletal muscle plasticity, including energy metabolism, protein degradation, redox homeostasis,

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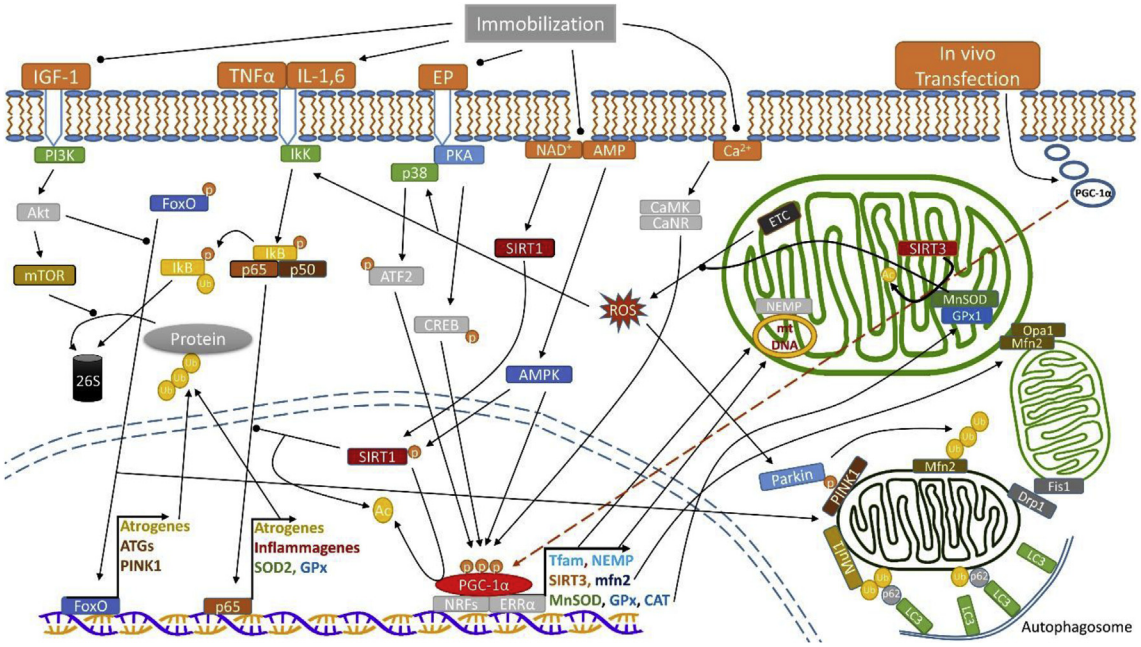

Fig. 1. Illustration of the effects of muscle immobilization on intracellular signaling pathways. Arrow-headed lines represent activation; dot-ended lines represent inhibition. See text for abbreviations.

apoptosis and muscle regeneration.[15] Specific to muscle disuse atrophy, FoxO3 controls both the ubiquitin-proteasome pathway and the autophagy-lysosome pathway, independently. As muscle stops contraction, diminished PI3K-Akt-mTOR signaling causes dephosphorylation of FoxO3, resulting in its nuclear sequestration and DNA binding.[16] Meanwhile, low energy state activates AMPK, which phosphorylates Ser-413 and -588 of FoxO3a, promoting its nuclear retention.[17] Thus, FoxO3 activation increases transcriptional activity of Atrogin-1 and MuRF-1 shown in the FoxO3 transgenic animals, whereas FoxO3 knockdown prevents muscle atrophy.[18] We previously reported that three weeks of IM decreased phospho-FoxO3a level in the tibialis anterior (TA) muscle severely, along with a 10-fold increase in Atrogin-1 mRNA and 4-fold increase in MuRF1 mRNA,[19] whereas Atrogin-1 and MuRF1 protein levels were elevated by 4- and 2.5-fold, respectively.[20] Besides FoxO, calpain and caspase-3 are important enzymes required for IM-induced muscle atrophy.[21] Moreover, RM also activates NFκB, a well-documented modulator of Atrogin-1 gene expression (Fig. 1).[14,20]

Muscle IM represents a catabolic state characterized by a rapid reduction of muscle mass, fiber size and number.[6,7] For example, after two weeks of IM, mouse TA and gastrocnemius muscles showed a 20–25% decrease in muscle-to-body weight ratio.[14] TA muscle cross-sectional area was almost down to 50% compared to its contralateral controls.[20] Importantly, metabolic functions of IM muscle were severely downregulated, demonstrated by the Krebs Cycle enzyme marker citrate synthase (CS), electron transport chain complex 4 (COXIV) activity, and ATP production rate.[20] Further, there is evidence that muscle fiber number can decrease after IM caused by enhanced apoptotic activity, such as increased BAX/Bcl2 ratio and caspase-3 activity.[19] Undoubtedly, reduced muscle size due to decreases of both myocyte number and size and deteriorated metabolic capacity can hamper muscle contractile function shown by compromised force production and calcium handling, as well as increased fatigability.[22] Thus, it is clear that cessation of muscle contractile activity over a few weeks can result in significant disturbance to the homeostasis of myocytes leading to enzymatic dis-regulation, proteolysis, and functional decline. We view these changes as the reversal of muscle hormesis and proposed to use the term "dehormesis". The question is, what is the cellular and molecular mechanism causing the dehormesis.

Critical role of mitochondrial homeostasis

Just as in the case that mitochondria play an important role in muscle hormetic adaptation to exercise,[3] loss of mitochondrial homeostasis (mitostasis) can be a primary reason for observed muscle morphological and functional defects after an extended period of disuse.[23] In contrast to an outdated view, the mitochondrion serves multi-functional roles far beyond being the "energy plant" of the cell. In all experimental models of studying muscle atrophy, one prominent observation was a reduction of mitochondrial volume. After two week of IM, TA muscle showed a 50% decrease in mitochondrial density,[19,20] with a further 50% loss in the third week.[20] It is noteworthy that 5 days of RM only recovered half of the reduced mitochondrial density. Considering the reduction of muscle mass during the corresponding time, the total reduction of mitochondrial quantity is devastating. There is some evidence that the majority of loss was the subsarcolemmal mitochondrial subpopulation.[24,25] In addition to the quantity change, mitochondrial quality was also severely affected shown by parallel decreases of CS and cytochrome c oxidase (COX) activities.[19] A dramatic decline of ATP production rate (per unit of mitochondrial protein) of more than 50% indicated that the muscle was energy deficient, whereas its implication in other vital muscle functions requiring ATP cannot be underestimated.

Skeletal muscle mitochondrion has a half-life of approximately two weeks, therefore the decline of its volume can be caused by either a decrease of biosynthesis or an acceleration of degradation, or both. Mitochondrial biogenesis is controlled primarily by PGC-1α, coactivation of which promotes the expression of Nrf-1 and Nrf-2, a key step for the gene express of nuclear-encoded mitochondrial proteins, and of Tfam, the key regulator of mitochondrial DNA (mtDNA) biosynthesis.[26] There is strong evidence that PGC-1α is downregulated during muscle IM, shown by reduction of both its mRNA and protein content in the nucleus and wholes muscle.[14,20,27,28] Nrf-1 content was suppressed to less than half of the control level while cytosolic and mitochondrial Tfam was also decreased.[20] These findings undoubtedly explain decreased mitochondrial oxidative capacity and mtDNA seen in the IM muscle. Whether or not downregulation of PGC-1α is the initial trigger for the decline of mitochondrial biogenesis still requires verification, as PGC-1α itself is also subjected to transcriptional and post-translational regulation by other signaling pathways.[29]

In an atrophying muscle, decreased mitochondrial quality and quantity can also be influenced by the organelle's degradation, which in turn is controlled by mitochondrial autophagy (mitophagy) and the fusion and fission dynamics.[19,30,31] Activation of mitophagy may be viewed as a protective mechanism, because accumulation of damaged mitochondria may stimulate apoptosis by membrane permeabilization and release of cytochrome c thus activating caspase-3.[32] A decline of mitochondrial inner membrane potential ($\Delta\psi m$) may serves as the initial signal for the expression of PINK1, which phosphorylates mitochondrial fusion protein-2 (Mfn2) as the docking point for Parkin, an ubiquitin ligase.[33] Beclin 1, BCL2/Adenovirus E1B 19 kDa Interacting Protein 3 (Bnip3), microtubule-associated protein 1 light chain 3 (LC3) and the autophagy adaptor protein p62/SQSTM1 (p62) are key players for forming the autophagosome, a necessary step for mitochondrial degradation.[33] In skeletal muscle, Beclin 1 and Bnip3/nix upregulation are controlled by FoxO.[34] Activation of this PINK1-Mfn2-Parkin axis facilitates the removal of damaged mitochondria to maintain a healthier mitochondrial pool, but decreases overall mitochondrial population in the disused muscle. After two weeks of IM followed by RM, both PINK1 and Parkin expressions were increased by several fold, whereas mitochondrial ubiquitin ligase Mul1 level was dramatically elevated.[19,35] These changes coincided with a plunge of Mfn2 level but an increase in Fis-1 expression. The finding that ubiquitinated Mfn2 and mitochondria were more than doubled in mouse TA muscle after IM-RM seems consistent with the overall upregulation of mitophagic enzymes including PINK1, Parkin, Beclin1, Bnip3 and LC3 II/I ratio.[35] Although increased mitophagy during IM may be a double-edged sword due to decreased mitochondrial quantity, keeping a smaller but more intact mitochondrial population is crucial for the muscle.

There is strong evidence that alteration of mitochondrial morphological changes due to fusion and fission protein expressions could affect many vital cellular functions and are critical to mitostasis.[36] Mfn2 repression in L6E9 muscle cells was shown to decrease the rate of pyruvate and glucose oxidation, reduce mitochondrial membrane potential ($\triangle\psi_m$) and cause a dramatic discontinuity of the mitochondrial network.[37] Interestingly, FoxO activation during muscle atrophy promotes the expression of Mul1, thus ubiquinating and degrading Mfn2.[38] Furthermore, Mfn1 and Mfn2 are also substrates for Parkin.[39] Parkin's substrates also include other important proteins such as mitochondrial Pho GTPase, membrane translocase (TOM70, TOM40 and TOM20) and voltage-dependent anion channel proteins (VDAC).[33] Decreased fusion and increased fission protein expression can make mitochondria more fragmented and easier to be isolated for removal by mitophagy.[32]

Disruption of redox signaling in disused muscle

In actively contracting skeletal muscle, PI3K-Akt-mTOR pathway phosphorylates and inactivates FoxO, thereby inhibiting ubiquitin-proteolysis and mitophagy. Contraction-mediated PGC-1α signaling and mitochondrial biogenesis maintain a healthy mitochondrial turnover and keep FoxO in check via its phosphorylation.[40] Furthermore, PGC-1α regulates intracellular redox status by reducing ROS generation (due to a healthier mitochondrial population) and upregulating antioxidant enzymes.[20,41] This homeostatic balance can be disrupted within the disused muscle. First, IM unmistakably damages mitochondrial membrane integrity, shown by enhanced mitochondrial lipid peroxidation, decreased membrane potential and ubiquitination.[20] As a result, increased ROS generation and oxidative stress take place in the IM muscle, especially in the mitochondria, evidenced by 8-isoprostane and 4-hydroxynonenoal accumulation. Secondly, during muscle IM, diminished PI3K-Akt-mTOR pathway causes dephosphorylation of FoxO3, resulting in its nuclear sequestration and DNA binding.[15,16] Additionally, activated AMPK during muscle IM phosphorylates Ser-413 and -588 of FoxO3a, promoting its nuclear retention.[17] AMPK is also a well-known activator of ULK-1 that initiates the early events of mitophagy.[29] As mentioned before, FoxO3 activation increases transcriptional activity of Atrogin-1 and MuRF-1.[18,42] Thirdly, there is strong evidence that prolonged IM and RM can activate NFκB signaling and increase production of pro-inflammatory cytokines and myokines such as TNF-α, IL-1β, IL-6, and MCP.[20,43,44] Both TNF-α and IL-1β are known stimulators of ROS generation from mitochondrial electron transport chain and other oxidases such as NADPH oxidase, COX-2 and lipoxygenase, thus escalating oxidative stress via a vicious cycle.

A good example that demonstrates disruption of redox signaling causing muscle disuse atrophy is the activation of apoptosis. PINK1/Mfn2/Parkin axis-induced mitochondrial ubiquitination, fragmentation and mitophagic degradation during IM leads to differential expression of Bcl-2 family proteins involved in both autophagy and apoptosis.[33] Muscle IM has been reported to increase the relative content of Bax (Bax/Bcl2 ratio), associated with the activation of caspase-3.[19] BNIP3, the proapototic protein, was clearly upregulated with IM-RM, when increased ROS and inflammation prevailed.[19,35] Bax is required for BNIP3-induced loss of mitochondrial inner membrane potential ($\triangle\psi_m$), which further destabilize mitochondrial membrane and enhance mitophagic tendency.[45] Furthermore, BNIP3 can induce cell death through atypical apoptosis without caspase-3 and cytochrome c release.[45] These findings suggest that mitochondrial dynamics change and mitophagy are closely related to muscle cell death. Thus, besides decreased myocyte cross-section area (sign of atrophy), myocyte number may also be reduced due to apoptosis, although an unequivocal conclusion on this matter still requires more investigation.

Amelioration of muscle atrophy using DNA transfection

Since muscle wasting is a serious threat to humans suffering from hospitalization, bedrest due to injury, denervation and microgravity, great interests have been generated to develop strategies to prevent this pathophysiological disorder. Experimental models to inhibit proteolytic, autophagic and apoptotic pathways include FoxO gene knockout,[9] inhibition of NFκB,[46] antioxidant intervention and transgenic overexpression of PGC-1α.[28,47] Over the past five years, we have adopted a technique to overexpress PGC-1α in mouse muscle via in vivo DNA injection followed by electroporation.[20] This method has been proven to be simple, efficient and safe, and produced valuable data to help understand not only the role of PGC-1α in ameliorating muscle disuse atrophy but also potential utility in clinical application. The details of the procedures are described in our recent publications.[48,49]

The first and most significant effect of PGC-1α transfection is the restoration of PGC-1α content in the cytoplasm, nucleus and mitochondria.[20] Aged mouse TA muscle also robustly overexpress PGC-1α after in vivo transfection.[35,48] As a result, mitochondrial density and mtDNA content were both elevated in the transfected TA muscle, along with higher levels of Tfam, suggesting these improvements were probably caused by increased mitochondrial proliferation. Importantly, mitochondrial oxidative function showed impressive enhancement such as increased CS, COXIV activity, and ATP production rate. These findings were in general agreement with data from transgenic animal studies overexpressing PGC-1α.[46,47]

PGC-1α local transfection decreased muscle oxidative stress, such as lipid and protein oxidative damage.[20] The protection may stem from two reasons, reduced ROS generation in the mitochondria and increased antioxidant defense.[20] Enhanced mitochondrial biogenesis leads to a "younger" and healthier mitochondrial population and hence less inner membrane defects, which is a main source of ROS generation. Increased PGC-1α reduced acetylation of mitochondrial SOD (SOD2), making it more active. The protection was caused by PGC-1α-induced upregulation of SIRT3, a mitochondrial deacetylase. Other mitochondrial enzymes were shown to be protected from acetylation due to SIRT3 upregulation.[50] PGC-1α transfection also increased muscle glutathione peroxidase (GPx) and catalase activities that control hydrogen peroxide concentration in the IM muscle.[20,48] Another major consequence of PGC-1α overexpression was the inhibition of IM-induced muscle inflammatory

response shown by NFκB inhibition and decreased expression of TNFα, IL-1β and IL-6.

Our recent findings that IM-activated mitophagy was suppressed by PGC-1α in both young and aged mouse muscle shed some additional lights on the protective mechanism of PGC-1α transfection.[19,48] Major players of mitophagy such as PINK1, Parkin, Mul1 and LC3II were upregulated in IM-RM muscle as previously mentioned, but were downregulated by PGC-1α transfection, together with a clear suppression of FoxO. As a strong supporting evidence, mitochondrial ubiquitination level was attenuated by PGC-1α. It is interesting to note that PGC-1α also mitigated mitochondrial fission protein (Fis-1 and Drp-1) expression in the aged muscle. This finding suggests that ameliorated mitochondrial dynamic changes by PGC-1α may prevent mitochondrial fragmentation, a main reason for higher mitophagy rate. It is noteworthy that although increased apoptosis was found in muscle disuse atrophy, PGC-1α transfection did not block this effect, suggesting other signaling pathways not related to mitochondrial quality control, such as calpains, may be involved.

The primary limitation of PGC-1α transfection method is the delivery of this nuclear cofactor.[49] Since the efficiency of DNA replication is diffusion-limited, research up to date has primarily used muscles with small size and proximal to body surface. Usage of electroporation helps DNA diffusion, but deep sections of the muscle still has low access to PGC-1α –rich plasmid. In mouse TA muscle, approximately 70% of the muscle fibers showed increased PGC-1α content (with immunostaining method), whereas in immobilized and transfected fibers a 25% increase of fiber cross-sectional area was found.[20] Future research may focus on the delivery system that can apply this technique to a wider range of muscles that suffer from disuse atrophy.

Conclusion

Skeletal muscle disuse atrophy represents a pathophysiological disorder characterized by excessive proteolysis and associated functional defects. This catabolic state may reflect the organism's adaptation to a low energy availability milieu for survival. While activation of FoxO is the initiator for the orchestrated protein degradation, loss of mitochondrial homeostasis plays a critical role in escalating the situation when decreased mitochondrial biogenesis, disrupted fusion-fission dynamics, increases ROS generation and inflammation lead to enhanced mitophagy and eventually apoptosis. Local overexpression of PGC-1α via DNA transfection has proven to effectively reverse the aforementioned events and significantly reduce myocyte atrophy.

Each authors' contributions

Li Li Ji is responsible for the research design, data analysis and manuscript writing and editing. Dongwook Yeo is responsible for experiment, data analysis, manuscript writing.

Submission statement

This manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

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References

1. Ji LL, Gomez-Cabrera MC, Vina J. Exercise and hormesis: activation of cellular antioxidant signaling pathway. *Ann N Y Acad Sci*. 2006;1067:425–435.
2. Radak Z, Chung HY, Goto S. Exercise and hormesis: oxidative stress-related adaptation for successful aging. *Biogerontology*. 2005;6:71–75.
3. Ji LL, Kang C, Zhang Y. Exercise-induced hormesis and skeletal muscle health. *Free Radic Biol Med*. 2016;98:113–122.
4. Peake JM, Markworth JF, Nosaka K, Raastad T, Wadley GD, Coffey VG. Modulating exercise-induced hormesis: does less equal more? *J Appl Physiol*. 2015;119:172–189.
5. Timmons JA, Norrbom J, Scheele C, Thonberg H, Wahlestedt C, Tesch P. Expression profiling following local muscle inactivity in humans provides new perspective on diabetes-related genes. *Genomics*. 2006;87:165–172.
6. Kandarian SC, Jackman RW. Intracellular signaling during skeletal muscle atrophy. *Muscle Nerve*. 2006;33:155–165.
7. Jackman RW, Kandarian SC. The molecular basis of skeletal muscle atrophy. *Am J Physiol Cell Physiol*. 2004;287:C834–C843.
8. Bodine SC, Latres E, Baumhueter S, et al. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science*. 2001;294(80-):1704–1708.
9. Gomes MD, Lecker SH, Jagoe RT, Navon A, Goldberg AL. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc Natl Acad Sci U S A*. 2001;98:14440–14445.
10. Clarke BA, Drujan D, Willis MS, et al. The E3 Ligase MuRF1 degrades myosin heavy chain protein in dexamethasone-treated skeletal muscle. *Cell Metabol*. 2007;6:376–385.
11. Kedar V, McDonough H, Arya R, Li HH, Rockman HA, Patterson C. Muscle-specific RING finger 1 is a bona fide ubiquitin ligase that degrades cardiac troponin I. *Proc Natl Acad Sci U S A*. 2004;101:18135–18140.
12. Li HH, Kedar V, Zhang C, et al. Atrogin-1/muscle atrophy F-box inhibits calcineurin-dependent cardiac hypertrophy by participating in an SCF ubiquitin ligase complex. *J Clin Investig*. 2004;114:1058–1071.
13. Tintignac LA, Lagirand J, Batonnet S, Sirri V, Leibovitch MP, Leibovitch SA. Degradation of MyoD mediated by the SCF (MAFbx) ubiquitin ligase. *J Biol Chem*. 2005;280:2847–2856.
14. Kang C, Ji LL. Muscle immobilization and remobilization downregulates PGC-1alpha signaling and the mitochondrial biogenesis pathway. *J Appl Physiol*. 2013;115:1618–1625.
15. Sanchez AM, Candau RB, Bernardi H. FoxO transcription factors: their roles in the maintenance of skeletal muscle homeostasis. *Cell Mol Life Sci*. 2014;71:1657–1671.
16. Bonaldo P, Sandri M. Cellular and molecular mechanisms of muscle atrophy. *Dis Model Mech*. 2013;6:25–39.
17. Greer EL, Oskoui PR, Banko MR, et al. The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. *J Biol Chem*. 2007;282:30107–30119.
18. Sandri M, Sandri C, Gilbert A, et al. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell*. 2004;117:399–412.
19. Kang C, Yeo D, Ji LL. Muscle immobilization activates mitophagy and disrupts mitochondrial dynamics in mice. *Acta Physiol*. 2016;218.
20. Kang C, Goodman CA, Hornberger TA, Ji LL. PGC-1alpha overexpression by in vivo transfection attenuates mitochondrial deterioration of skeletal muscle caused by immobilization. *FASEB J*. 2015;29:4092–4106.
21. Talbert EE, Smuder AJ, Min K, Kwon OS, Powers SK. Calpain and caspase-3 play required roles in immobilization-induced limb muscle atrophy. *J Appl Physiol*. 2013;114:1482–1489.
22. Eshima H, Miura S, Senoo N, Hatakeyama K, Poole DC, Kano Y. Improved skeletal muscle Ca(2+) regulation in vivo following contractions in mice overexpressing PGC-1alpha. *Am J Physiol Regul Integr Comp Physiol*. 2017;312:R1017–R1028.
23. Romanello V, Sandri M. Mitochondrial biogenesis and fragmentation as regulators of muscle protein degradation. *Curr Hypertens Rep*. 2010;12:433–439.
24. Adhihetty PJ, Ljubicic V, Menzies KJ, Hood DA. Differential susceptibility of subsarcolemmal and intermyofibrillar mitochondria to apoptotic stimuli. *Am J Physiol Cell Physiol*. 2005;289:C994–C1001.
25. Krieger DA, Tate CA, McMillin-Wood J, Booth FW. Populations of rat skeletal muscle mitochondria after exercise and immobilization. *J Appl Physiol Respir Environ Exerc Physiol*. 1980;48:23–28.
26. Wu Z, Puigserver P, Andersson U, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell*. 1999;98:115–124.
27. Cannavino J, Brocca L, Sandri M, Bottinelli R, Pellegrino MA. PGC1-alpha over-expression prevents metabolic alterations and soleus muscle atrophy in hindlimb unloaded mice. *J Physiol*. 2014;592:4575–4589.
28. Sandri M, Lin J, Handschin C, et al. PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. *Proc Natl Acad Sci U S A*. 2006;103:16260–16265.
29. Kim Y, Triolo M, Hood DA. Impact of aging and exercise on mitochondrial quality control in skeletal muscle. *Oxid Med Cell Longev*. 2017;2017:3165396.
30. Harman D. The free radical theory of aging. *Antioxidants Redox Signal*. 2003;5:557–561.
31. Sakellariou GK, Pearson T, Lightfoot AP, et al. Mitochondrial ROS regulate oxidative damage and mitophagy but not age-related muscle fiber atrophy. *Sci Rep*. 2016;6:33944.
32. Green DR, Van Houten B. SnapShot: mitochondrial quality control. *Cell*. 2011;147,950, 950 e1.
33. Palikaras K, Tavernarakis N. Mitochondrial homeostasis: the interplay between mitophagy and mitochondrial biogenesis. *Exp Gerontol*. 2014;56:182–188.
34. Mammucari C, Milan G, Romanello V, et al. FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metabol*. 2007;6:458–471.
35. Kang C, Ji LL. PGC-1alpha overexpression via local transfection attenuates mitophagy pathway in muscle disuse atrophy. *Free Radic Biol Med*. 2016;93:32–40.
36. Chan DC. Mitochondrial fusion and fission in mammals. *Annu Rev Cell Dev Biol*. 2006;22:79–99.

37. Bach D, Pich S, Soriano FX, et al. Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. *J Biol Chem*. 2003;278:17190–17197.
38. Lokireddy S, Wijesoma IW, Teng S, et al. The ubiquitin ligase Mul1 induces mitophagy in skeletal muscle in response to muscle-wasting stimuli. *Cell Metabol*. 2012;16:613–624.
39. Gegg ME, Cooper JM, Chau KY, Rojo M, Schapira AH, Taanman JW. Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. *Hum Mol Genet*. 2010;19:4861–4870.
40. Sanchez AMJ, Candau RB, Bernardi H. FoxO transcription factors: their roles in the maintenance of skeletal muscle homeostasis. *Cell Mol Life Sci*. 2014;71:1657–1671.
41. Schreiber SN, Emter R, Hock MB, et al. The estrogen-related receptor alpha (ERRalpha) functions in PPARgamma coactivator 1alpha (PGC-1alpha)-induced mitochondrial biogenesis. *Proc Natl Acad Sci U S A*. 2004;101:6472–6477.
42. Stitt TN, Drujan D, Clarke BA, et al. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Mol Cell*. 2004;14:395–403.
43. Guttridge DC, Mayo MW, Madrid LV, Wang CY, Baldwin Jr AS. NF-kappaB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. *Science*. 2000;289(80-):2363–2366.
44. Kang C, Shin WS, Yeo D, Lim W, Zhang T, Ji LL. Anti-inflammatory effect of avenanthramides via NF-kappaB pathways in C2C12 skeletal muscle cells. *Free Radic Biol Med*. 2018;117:30–36.
45. Zhang J, Ney PA. Role of BNIP3 and NIX in cell death, autophagy, and mitophagy. *Cell Death Differ*. 2009;16:939–946.
46. Handschin C, Chin S, Li P, et al. Skeletal muscle fiber-type switching, exercise intolerance, and myopathy in PGC-1alpha muscle-specific knock-out animals. *J Biol Chem*. 2007;282:30014–30021.
47. Wenz T, Rossi SG, Rotundo RL, Spiegelman BM, Moraes CT. Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging. *Proc Natl Acad Sci U S A*. 2009;106:20405–20410.
48. Yeo D, Kang C, Gomez-Cabrera MC, Vina J, Ji LL. Intensified mitophagy in skeletal muscle with aging is downregulated by PGC-1alpha overexpression in vivo. *Free Radic Biol Med*. 2019;130.
49. Yeo D, Kang C, Gomez-Cabrera MC, Vina J, Ji LL. Data on in vivo PGC-1alpha overexpression model via local transfection in aged mouse muscle. *Data Br*. 2019;22.
50. Baeza J, Smallegan MJ, Denu JM. Mechanisms and dynamics of protein acetylation in mitochondria. *Trends Biochem Sci*. 2016;41:231–244.