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Precision remodeling: how exercise improves mitochondrial quality in myofibers

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Abstract

The profound energetic demand of prolonged exercise imposed upon skeletal muscle and the heart is met by oxidation of substrate within mitochondria. As such, several coordinated events are initiated in order to maintain mitochondria, collectively known as mitochondrial quality control. In this review, we discuss how mitochondrial quality control functions to maintain the integrity of the reticulum and energy production in response to prolonged exercise, as well as the relevant signaling events that dictate these responses. Based upon the prevailing data in the field, we propose a model where exercise-mediated quality control may be chiefly regulated through local mechanisms, thus allowing for the remarkable precision in mitochondrial quality control events.

Keywords

exercise; muscle; myofiber; mitochondria; mitophagy; quality control

1. Myofiber mitochondria: function follows form

Skeletal muscle and heart consist primarily of specialized cells called myofibers, which are capable of generating mechanical force through contraction. Force produced by skeletal muscles facilitates movement, ranging from activities of daily living to feats of athleticism while increased force production in the heart assists other peripheral tissue function, including skeletal muscles, by increasing blood flow, thus providing more oxygen and

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metabolic substrates for oxidation. Sufficient ATP production is tantamount to increased force productions in both skeletal and cardiac myofibers. Energy production can increase as much as 100 fold in skeletal muscle¹ and approximately 3 fold in the heart² during prolonged periods of increased contraction, as can occur during exercise. Such a profound increase in energetic demand during prolonged exercise is met by oxidation of metabolic substrates within mitochondria.

Mitochondria are double membrane organelles, consisting of an outer membrane (OMM), an inner membrane (IMM), and an interior region known as the matrix. OMM contains various transport proteins for import and export of protons, metabolic intermediates and substrates, whereas IMM forms an elaborate, folded membrane structure called cristae, which contains macromolecules of the electron transport chain proteins where oxidative phosphorylation of metabolic intermediates takes place. Mitochondrial matrix contains all the proteins and enzymes required for generation of ATP via the Krebs's Cycle as well as proteins and enzymes for other metabolic pathways. Myofiber mitochondria are particularly enriched in proteins involved in the TCA cycle, electron transport chain, and oxidative phosphorylation³⁻⁷, illustrating a physiologic safeguard for meeting energetic demands in these tissues.

In their native state, myofiber mitochondria form an intricate reticulum that extends along the length of the cell⁸⁻¹¹, which in humans can be in the tens of centimeters in length. Myofiber mitochondria function as a syncytium with potential energy distributed across the reticulum^{9,10,12}. Recent evidence suggests that morphology of the reticulum is directly related to the metabolic properties of a given myofiber. For example, glycolytic myofibers (e.g. plantaris muscle in the hindlimb), which rely primarily on glycolysis for ATP production, have a less robust mitochondrial reticulum among myofibrils and it interacts more with the sarcoplasmic reticulum, presumably owing to the high demands of Ca²⁺ cycling during repeated contractions¹¹. In contrast, mitochondrial reticulum in oxidative myofibers (e.g. soleus muscle in the hindlimb and the heart) take up approximately a third of cell volume and is geometrically associated with lipid droplets and the contractile proteins¹¹. Such phenotypic variation between myofiber reticula is thought to be optimal for tissue function, as contractile power is of greater importance in glycolytic muscle compared to preferential maintenance of energetic homeostasis in oxidative myofibers, such as the heart^{9,11}.

2. Mitochondrial quality control maintains both structure and function

The high degree of structure to function specialization in myofiber mitochondria implies that it is closely monitored and regulated to maintain energy production capacity. These processes that regulate the mitochondrial reticulum are collectively referred to as mitochondrial quality control. Mitochondrial quality control largely consists of biogenesis, dynamics (i.e. fission and fusion), and selective degradation via proteolysis and mitophagy. In a non-pathological state, these processes are temporally upregulated in response to prolonged energetic stress, such as exercise¹³⁻¹⁶. This upregulation during prolonged energetic stress accomplishes at least two goals: 1) ensures energetic production and homeostasis by the mitochondrial reticulum during high demand, and 2) orchestrates

adaptation of the reticulum to better meet similar energetic demands in the future. As we have discussed in depth elsewhere the various signaling factors involved in mitochondrial quality control pertaining to exercise^{15,16}, we will provide only an abridged discussion of the main processes here, though framed within the current context of mitochondrial reticulum structure and function.

2.1 Exercise-induced Mitochondrial Dynamics

One of the first events of mitochondrial quality control in skeletal muscle during prolonged exercise appears to be mitochondrial fission. This is evidenced in that phosphorylation of the fission protein, dynamin-related protein 1 (Drp1) at its activating S616 site is increased following acute exercise in both mice and humans^{13,14,17,18} and is likely activated early during a prolonged exercise bout¹⁴. *Ex vivo* evidence suggests that the mitochondrial reticulum can physically separate regions of itself within minutes⁹, though whether mitochondrial fission is activated with similar urgency under physiological stimuli is unknown, but not unreasonable to speculate. Fission of selected regions of mitochondrial reticulum serves both to acutely preserve the reticulum and to pave the way for degradation of selected mitochondria through mitophagy (discussed in subsequent section)^{15,16}. It is perhaps not surprising then that impaired fission in skeletal muscle, through muscle-specific deletion of Drp1, reduces exercise capacity¹⁴. Likewise, conditional deletion of Drp1 in the heart impairs not only mitochondrial respiratory function but also cardiac function¹⁹, as well as exacerbates the development of cardiac pathologies²⁰.

Inversely to fission, an adaptive response to periods of increased oxidative phosphorylation in myofibers is increased fusion of mitochondria to support exchange of matrix components^{21–23}, thus aiding energetic efficiency of the reticulum. Loss of fusion protein optic atrophy 1 (OPA1) impairs mitochondrial function *in vitro*¹⁴, and skeletal muscle-specific loss of the primary fusion proteins (Mitofusin 1 and 2) impairs exercise training-induced improvements in exercise capacity and impairs mitochondrial respiratory function in mice²⁵. Exercise training in humans, increases the fusion to fission protein ratio in skeletal muscle, suggesting a condition favoring a more fused mitochondria reticulum²⁶. Taken together, the capacity to precisely reorient the architecture of mitochondrial reticulum is essential for myofiber energetic homeostasis, with fission potentially more important during acute periods of stress and fusion more involved in the adaptive responses to exercise training.

2.2 Exercise-induced Mitophagy

A possible outcome for regions of mitochondrial reticulum to be separated from the whole via fission is degradation through mitophagy. Evidence from our lab in mouse skeletal muscle suggests that exercise-induced mitophagy is likely initiated during exercise but does not resolve (i.e. fusion of autophagosome containing mitochondria with lysosome) until well into the recovery period¹³. Upregulation of mitophagy in response to prolonged exercise is mediated through the energetic nucleotide sensor, 5' AMP-activated Protein Kinase (AMPK). While AMPK is involved in various metabolic processes related to energetic stress, in the context of mitophagy, activated AMPK phosphorylates Unc51-like autophagy activating kinase 1 (Ulk1) at Ser555^{13,27,28}. Phosphorylation at Ser555 activates Ulk1,

initiating the formation of the autophagosome through various downstream effectors^{15,29–31}. Loss of Ulk1 in skeletal muscle not only blocks mitophagy induced by acute exercise but also impairs metabolic adaptation to exercise training¹³. While exercise-induced mitophagy through Ulk1 in cardiac myofibers has not been described in the literature, Ulk1 has been recently proposed to be the predominant mechanism by which mitophagy protects cardiac myofibers from ischemia and starvation³².

While it is clear that Ulk1 is integral to the mitophagy response following acute exercise, the downstream effectors of Ulk1 are still being elucidated. One downstream substrate of Ulk1 that has emerged recently as a critical factor for energetic stress-induced mitophagy in myofibers is FUN14-domain containing 1 (Fundc1). When activated, Ulk1 translocates to mitochondria where it phosphorylates Fundc1 at Ser17, enhancing its binding capacity with the autophagosome membrane via microtubule-associated protein light chain 3 (LC3) *in vitro*³³. Skeletal muscle-specific loss of Fundc1 impairs mitochondrial energetics, exercise performance, and adipose metabolism³⁴. In *drosophila m.* knock-down of other suggested Ulk1 substrates (e.g. Atg2, Atg9, and Atg18) dramatically disrupts mitochondrial reticulum in indirect flight muscle and heart, resulting in poor tissue function and reduced lifespan³⁵. While much is still unknown how the downstream mitophagy process is mediated in response to acute exercise, it is clear that recognition and degradation of damaged and/or dysfunctional regions of mitochondrial reticulum is critical for tissue function, as well as systemic health, and this is a key component of how the beneficial effects of exercise are mediated.

2.3 Exercise-induced Mitochondrial Biogenesis

Finally, and arguably the most studied aspect of mitochondrial quality control in response to exercise in myofibers is mitochondrial biogenesis. Exercise is a potent inducer of the main transcriptional regulator of mitochondria, peroxisome proliferator-activated receptor gamma coactivated 1-alpha (PGC1 α), resulting in increased expression of mitochondria-related mRNA³⁶. Due to the energetic expense of prolonged exercise, mRNA translation is slowed to conserve ATP^{37, 38}, in part due to increased AMPK activity, presumably through its antagonistic action on protein synthesis by inhibiting the mechanistic target of rapamycin complex I (mTORC1)³⁹. However, long-term inhibition of mTORC1 through rapamycin feeding does not impair mitochondrial biogenesis in either skeletal muscle or heart^{40,41}, suggesting an alternative translational mechanism for the bulk of mitochondria-related mRNA. Presumably, once exercise is stopped and energetics return to a new homeostasis, bulk synthesis of mRNA resumes, and incorporation of newly translated proteins into mitochondrial reticulum is aided through fusion, leading to an expansion of the reticulum in myofibers¹⁵.

In summary, mitochondria are able to respond to energetic stress caused by acute exercise through a rapid change of their confirmation to maintain energetic output. Simultaneously, this remodeling of the reticulum aids in the removal and degradation of areas that would otherwise impede the capacity for mitochondria to match the energetic demands. Likewise, these events lead to adaptive changes in the structure of the reticulum to better meet future

energetic challenges. Therefore, exercise-regulated mitochondrial quality control illustrates the interdependency between the structure and the function in myofiber mitochondria.

3. Spatial specificity of mitochondrial quality control

Mitochondria in myofibers functions as a syncytium, with energy distributed across the reticulum^{9,10,12}. During exercise, mitochondrial function dramatically increases⁴². In agreement with an optimal distribution of potential energy across the reticulum, acute energetic stress as a result of different stimuli (including exercise) also appears to be uniform across mitochondrial reticulum^{13,43–15}. Interestingly though, advances in molecular imaging technology with the development of mitochondrial reporter genes (mt-Kiema⁴⁶, MitoTimer^{43,44}, and mitoQC⁴⁷) for visualizing mitochondrial quality control processes has revealed a high degree of spatial specificity in these processes. For example, acute exercise results in only a small percentage of the overall mitochondrial reticulum being targeted for degradation through mitophagy in skeletal muscle, evidenced by distinct mitochondria-originating puncta encased in autolysosomes^{13,43} (Fig. 1A and B). Similarly, acute ischemia or starvation also results in spatially distinct mitophagy events in the heart and skeletal muscle^{32,44,45}. Spatial specificity in mitophagy is in agreement with the spatial variability that is observed in fission events, a prerequisite for mitophagy, within myofibers^{9,21}. Furthermore, synthesis rates of mitochondrial proteins in the heart and skeletal muscle displays a great deal of heterogeneity^{48–50}, and turnover of the fluorescent mitochondria reporter MitoTimer in the heart is also spatially variable⁵¹. In that light, it is reasonable to speculate that biogenesis and incorporation of new mitochondria proteins in response to exercise occurs in a spatially heterogeneous manner as well.

Collectively, these observations suggest a model where, in a non-pathologic state, mitochondrial quality control is regulated by local mechanisms in distinct domains across the reticulum in myofibers (Fig. 1C). Given how closely mitochondrial quality control is related to mitochondrial energetics^{9,21}, it is possible that fluctuations in local energetics may dictate spatial mitochondrial quality control. For example, in the heart, Ca²⁺ influx and efflux are spatially controlled across the mitochondrial reticulum to maintain its overall function⁵². Thus, local failures in energy production, possibly resulting in separation of that domain(s) from the whole⁹, and setting off a cascade of local events (i.e. mitophagy and biogenesis/fusion) to restore homeostasis at the affected domain. However, potential signaling regulator(s) for such a local mitochondrial quality control domain response are unknown.

4. Conclusion: Importance of targeted remodeling for disease intervention.

The molecular response in mitochondrial quality control to acute exercise in a healthy or nonpathological state represents the physiological ideal stress signaling response. That is to say exercise elicits the appropriate changes to mitochondrial reticulum that maximize energetic output and, overtime, results in adaptations to the system to better meet future energetic challenges (i.e. training adaptations). Under disease conditions, however, poor mitochondria quality in myofibers is a common characteristic, as are impairments in aspects

of quality control mechanisms (i.e. aging, sarcopenia, neuromuscular and cardiovascular diseases)^{29,53–55}. Therefore, understanding how mitochondrial quality control is spatially mediated by energetic stress could lead to novel interventions to treat disease or aid in recovery from injury (e.g. myocardial infarction). Elucidating the mechanisms that dictate how specific regions are distinguished from the vast, complex mitochondrial reticulum is an important step in developing efficacious therapies to improve mitochondrial quality in chronic disease.

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* Of special interest

** Of outstanding interest

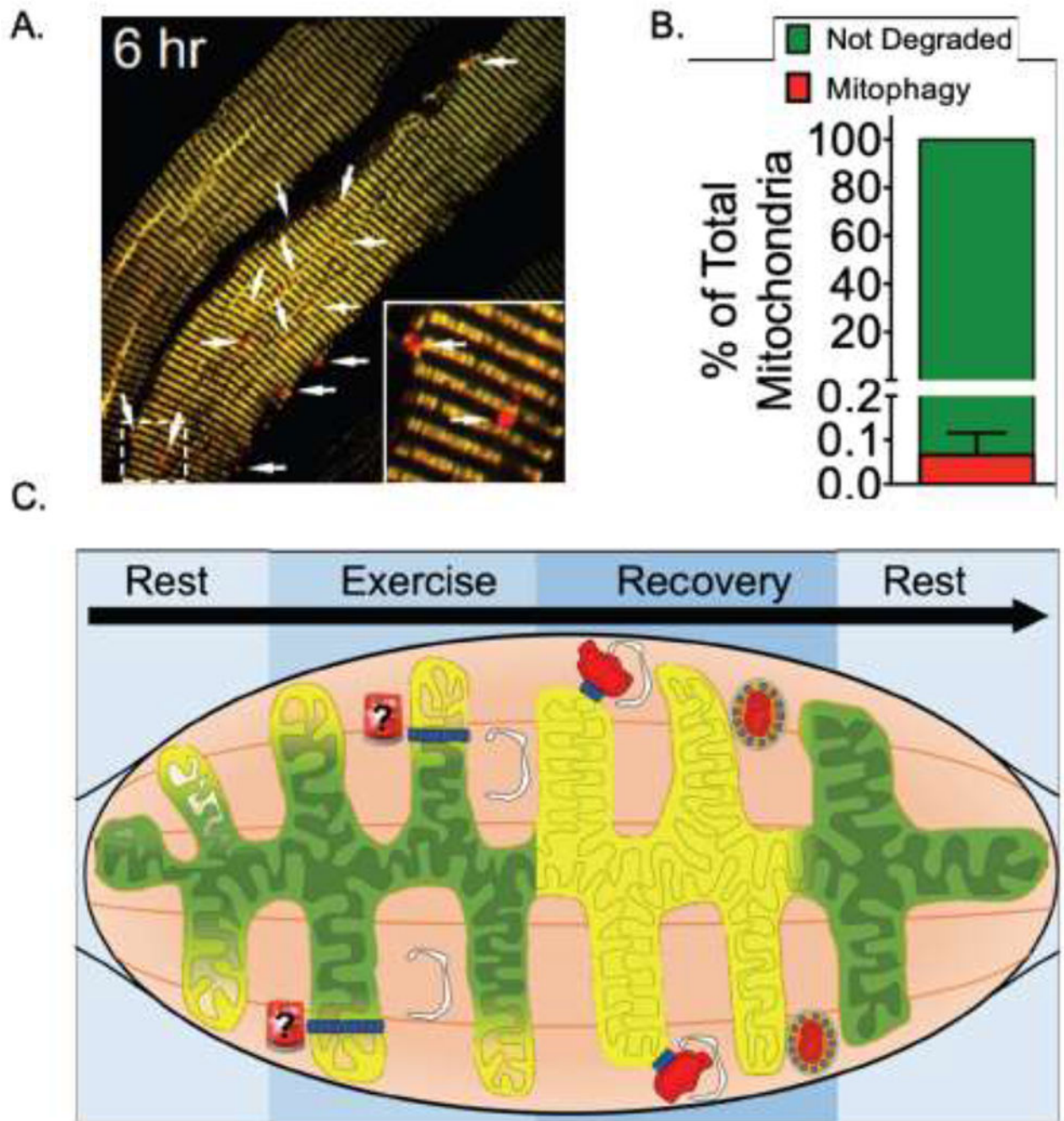
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**Figure 1.**

Model for domain-specific regulation of mitochondria quality control. A) Representative image of exercise-induced mitophagy in skeletal muscle (Laker and Drake, et al. Nat Commun. 2017, 8: 548). B) Quantification of mitochondria engulfed in autolysosomes (red puncta) relative to total mitochondrial area. Based on data published in Laker and Drake, et al. Nat Commun. 2017, 8: 548. While semi-quantitative, this calculation illustrates that the areas of the reticulum targeted for mitophagy in response to acute exercise is a small fraction

of the network. C) Illustration of local-mediation of mitochondria quality control to maintain energetic homeostasis and adaptation to energetic stress.

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