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ACTA PHYSIOLOGICA

RAPID COMMUNICATION

A single bout of resistance exercise triggers mitophagy, potentially involving the ejection of mitochondria in human skeletal muscle

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Abstract

Aim: The present study aimed to investigate the effects of a single bout of resistance exercise on mitophagy in human skeletal muscle (SkM).

Methods: Eight healthy men were recruited to complete an acute bout of one-leg resistance exercise. SkM biopsies were obtained one hour after exercise in the resting leg (Rest-leg) and the contracting leg (Ex-leg). Mitophagy was assessed using protein-related abundance, transmission electron microscopy (TEM), and fluorescence microscopy.

Results: Our results show that acute resistance exercise increased pro-fission protein phosphorylation ($DRP1^{Ser616}$) and decreased mitophagy markers such as PARKIN and BNIP3L/NIX protein abundance in the Ex-leg. Additionally, mitochondrial complex IV decreased in the Ex-leg when compared to the Rest-leg. In the Ex-leg, TEM and immunofluorescence images showed mitochondrial cristae abnormalities, a mitochondrial fission phenotype, and increased mitophagosome-like structures in both subsarcolemmal and intermyofibrillar mitochondria. We also observed increased mitophagosome-like structures on the subsarcolemmal

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cleft and mitochondria in the extracellular space of SkM in the Ex-leg. We stimulated human primary myotubes with CCCP, which mimics mitophagy induction in the Ex-leg, and found that BNIP3L/NIX protein abundance decreased independently of lysosomal degradation. Finally, in another human cohort, we found a negative association between BNIP3L/NIX protein abundance with both mitophagosome-like structures and mitochondrial cristae density in the SkM.

Conclusion: The findings suggest that a single bout of resistance exercise can initiate mitophagy, potentially involving mitochondrial ejection, in human skeletal muscle. BNIP3L/NIX is proposed as a sensitive marker for assessing mitophagy flux in SkM.

KEYWORDS

BNIP3L/NIX, mitochondria cristae, mitochondria dynamics, mitophagy

1 | INTRODUCTION

The human skeletal muscle (SkM), which accounts for approximately 40% of body mass in humans, is a highly active metabolic tissue that plays a crucial role in insulin-stimulated glucose disposal[1] and in locomotion. Mitochondria are essential players in SkM metabolism and function, and their dysfunction or morphology alteration has been associated with lipotoxicity and insulin resistance,[2,3] as well as sarcopenia and dystrophy in humans.[4,5] Exercise training is a cornerstone for the improvement of SkM metabolism and the prevention of metabolic diseases.[6] In particular, resistance exercise provides a potent anabolic stimulus to increase SkM mass and strength.[7] However, the effects of resistance exercise on mitochondrial function and morphology are not yet fully understood and remain controversial.[8] Previous research has shown that resistance exercise training increases mitochondrial respiratory capacity in human SkM without increasing citrate synthase activity as a marker of mitochondrial density.[9–11] Moreover, resistance-trained individuals possess an increased mitochondrial cristae density[12] – a marker of metabolic capacity.[2,13] Together, these studies suggest that resistance exercise training may enhance the mitochondrial oxidative capacity of SkM, potentially by remodeling the mitochondrial ultrastructure without affecting mitochondrial density. However, the underlying mechanism remains unknown.

Mitochondria are dynamic organelles that adapt their morphology and function in response to intracellular and extracellular stimuli.[14] Their morphology depends on the balance of the two interrelated processes called mitochondrial fusion and mitochondrial fission. Mitochondrial fusion is regulated by mitofusins 1 and 2 (MFN1/2) and optic atrophy 1 protein (OPA1), connecting outer and inner mitochondrial membranes, respectively.[15] Fusion supports multiple elements of mitochondrial biology such as mtDNA integrity, mitochondrial respiration, and apoptosis. Mitochondrial fission is regulated by dynamin-1-like protein (DRP1), which generates a constricting ring around mitochondria and drives its division.[16] Fission is required to segregate damaged mitochondria for mitophagy[17] – a vital quality control mechanism to preserve a healthy mitochondrial pool[18] that is activated when mitochondria present low membrane potential or irreversible mtDNA damage.[17,19] So far, two different mechanisms have been described to regulate mitophagy. The first is ubiquitin-dependent and comprises the phosphatase and tensin homolog (PTEN)-induced putative kinase protein 1 (PINK1) – Parkin pathway. The second is ubiquitin-independent, or receptor-mediated, and comprises the proteins BCL2/adenovirus E1B 19-kDa interacting protein 3 (BNIP3), BNIP3-like (BNIP3L/NIX),[20] and FUN14 domain-containing 1 (FUNDC1).[21,22] Both generate an "eat me" signal to engulf the damaged mitochondria into a mitophagosome. The mitophagosome then fuses with the lysosome, resulting in the degradation of the mitochondria into a mitolysosome. However, multiple mitochondrial quality control pathways have been described. A recent study found that damaged mitochondria can be ejected from cardiomyocytes via extracellular vesicles (EVs).[23] A decrease in the mitophagy process induces an accumulation of damaged mitochondria in SkM and SkM deterioration in mice.[24] As such, mitophagy could be considered an indispensable mechanism for quality control that could affect mitochondrial oxidative capacity adaptations following resistance exercise in human SkM.

In humans, 8 weeks of endurance training increased the abundance of mitophagy proteins, suggesting an

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