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Fis1 governs normal mitophagy in slow muscle during the low-intensity and long-period exercise

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Objective Mitochondrial dynamics include mitochondrial fusion and mitochondrial fission. It has long been widely recognized that Fis1 plays a role in mitochondrial fission in mammals. However, the finding of Dr. Youle's team suggests that Fis1 may play an important role in mediating normal mitophagy. Both of mitochondrial dynamics and mitophagy are closely related to skeletal muscle homeostasis. Therefore, in this study, Fis1 was specifically knocked out in skeletal muscle in vivo, looking forward to: 1) investigating the relationship between Fis1 and mitochondrial morphology, mitophagy in mouse skeletal muscle. 2) The mechanism of Fis1 in mitochondrial quality in skeletal muscle under exercise stress. So as to we can clarify the molecular mechanism of Fis1 in mediating mitochondrial quality in skeletal muscle, but also expect to provide more theoretical basis for skeletal muscle health and exercise adaptation.
Methods We constructed conditional skeletal muscle Fis1 knockout mice (C57BL/6) and littermate control mice through Cre / Loxp technique. The mice were free feeding, drinking and activity during the teat, we only selected male mice for all of the tests. And the genotypes were $Fis1^{FL/FL}$ MCK-Cre + (Fis1KO) and $Fis1^{FL/FL}$ MCK-Cre – (WT). First, we performed endurance test on 10 WT and 10 Fis1KO mice (32-40 weeks, n = 10), then dissected quadriceps, gastrocnemius and soleus (n=4-5) quickly and rapidly frozen in liquid nitrogen, then stored at -80 ° C freezer for testing Fis1 and OXPHOS expression (Western-blot). On the other hand, we selected WT and Fis1KO mice (n=3) to prepare EM samples, so as to observe mitochondrial morphology and muscle ultrastructure. Skeletal muscle (n=3-4) was snap-frozen in isopentane cooled with liquid nitrogen for HE, NADH staining, and observing GFP-LC3 (mitophagy).
Base on the exploration of loss of Fis1 without stress, we adopted endurance exhaustive exercise on WT (WT EEE) and Fis1KO mice (Fis1KO EEE) (n=3-4). Mice were acclimated to and trained on a 10^{o} uphill treadmill. Mice were acclimated to and trained on a 10^{o} uphill treadmill (Columbus Instruments) for 2 days. On day 3, mice were subjected to a single bout of running starting at the speed of 10m/min. Forty minutes later, the treadmill speed was increased at a rate of 1m/min every 10 min for a total of 30 min, and then increased at the rate of 1m/min every 5 min until mice were exhausted. Exhaustion was defined as the point at which mice spent more than 5 s on the electric shocker without attempting to resume running even if we used short air puffs and tail tickles with bristle brush. We dissected soleus and gastrocnemius to observe muscle ultrastructure and mitophagy through EM and confocal microscope respectively (same methods as before). At the same time, we used immune-EM to observe autophagosome morphology and LC3 distribution.
Results Behavior test on specific knock out skeletal muscle Fis1 mice model
We found that loss of Fis1 induced significantly lower performance in treadmill endurance tests than controls ($P < 0.001$).
The effect of loss of Fis1 on mitochondrial morphology and function
In soleus, knocking out Fis1 caused mitochondrial hyperfusion (mitochondrial size was significantly increased, $P = 0.01$). In addition, we found more swollen mitochondria in Fis1 knock-out gastrocnemius.

On the other hand, compared with the control mice, lack of Fis1 significantly reduced the protein expression of Complex I, Complex II and Complex IV in soleus ($P < 0.01$). As same as before, we also found a significant increase GFP-LC3 ($P < 0.01$) in Fis1KO soleus.
3) Changes of muscle ultrastructure and mitophagy after endurance exhaustive exercise (EEE)
First, comparing with the control group, swollen sarcoplasmic reticulum (skeletal muscle endoplasmic reticulum (ER)) and extremely swollen terminal cisternae (TC) were found in Fis1KO soleus and gastrocnemius respectively after endurance exhaustive exercise.
We found a significant accumulation of GFP-LC3 ($P < 0.0001$) in Fis1KO soleus compared to the control. However, GFP-LC3 signal still increased ($P < 0.001$) in Fis1KO soleus after exercise compared with that in soleus before exercise. Moreover, we observed a lot of large and irregular autophagosomes appeared in Fis1KO soleus after EEE through immune electron microscope.
Conclusions 1) Loss of Fis1 causes a certain degree of mitochondrial hyperfusion, increases mitophagy and significantly decreases mitochondrial function in slow muscle. However, losing Fis1 does not cause obvious alteration on quick muscle and synthetic muscle. Therefore, the absence of Fis1 has a significant effect on mitochondria-rich muscle.
2) Mitochondrial-ER interactions may be involved in the connection of endoplasmic reticulum swelling after endurance exhaustive exercise.
3) Endurance exercise with oxidative phosphorylation aggravate the increase and abnormality of mitophagy caused by the loss of Fis1 in slow muscle, suggesting that Fis1 governs normal mitophagy in slow muscle during the low-intensity and long-period exercise. This phenomenon may be related to the worse performance in treadmill endurance test of Fis1 KO mice.