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REVIEW

Effects of Eccentric Exercise on Skeletal Muscle Injury: From An Ultrastructure Aspect: A Review

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The contraction of skeletal muscle (SM) plays an important role in providing power for the body to participate in physical activities. Nevertheless, unaccustomed motions, especially eccentric exercises (EE), might cause changes in ultrastructure and mitochondria of SM, increasing potential risks of injuries. The objective of this narrative literature review was to summarize the effects of EE on SM ultrastructure. The results found that EE may lead to changes in the ultrastructure of SM, including the ambiguity of each line and band in the sarcomere, vacuolar degeneration of mitochondria, swelling of the sarcoplasmic reticulum, and disordered arrangement of myofibrils. At the same time, EE also leads to redistribution of SM mitochondria, changes in dynamics, and mitochondrial autophagy. This review provides additional insight into further research on the influence of EE on injuries of SM.

Keywords: Eccentric Exercise; Skeletal Muscle; Ultrastructure; Mitochondrion; Injury

Introduction

Skeletal muscle (SM) is the largest tissue in the human body, accounting for 40%–45% of body weight. SM contraction provides power for the body to participate in sports (Draper and Marshall, 2014). Performing unaccustomed exercises, especially eccentric exercise (EE), could cause SM damage and change its ultrastructure (Kristian et al., 2008, Sorichter et al., 1997). SM damage caused by EE will cause changes in the cytoskeleton that maintains the ultrastructure of SM in SM cells, and also lead to abnormal distribution, structure and function of mitochondria, and autophagy (Dong et al., 2014, Magalhães et al., 2009). EE can also cause endoplasmic reticulum stress in SM cells (Bruno et al., 2016).

Muscle cells, also called muscle fibers, are the basic structure and functional unit of muscle (Draper and Marshall, 2014). There is a thin layer of connective tissue membrane outside each muscle fiber, which is called endomysium. Many muscle fibers are arranged in bundles called muscle bundles. Many muscle bundles are arranged together to form a muscle, and the outer bread of the muscle has a connective tissue membrane, called epimuscular membrane (Draper and Marshall, 2014).

Each muscle fiber contains hundreds to thousands of myofibrils. Myofibrils are composed of thick and thin muscle filaments arranged in parallel. The length of the thick filaments is the length of the A-band (dark band). One end of the thin filament is connected to the Z-line, and one end is passed through the I-band, extending between two parallel thick filaments. There are only thin myofilaments in the I-band. The A-band is composed of thick and thin myofilaments. The thin myofilaments from the Z-line on both sides do not meet in the middle of the A-band. There is an area in which there are only thick myofilaments, which is the H-area. The middle of the H-area is called the M-line. The structure between two adjacent Z-lines is the basic structure and functional unit of muscle fibers, called sarcomere (Draper and Marshall, 2014).

Mitochondria exist in most eukaryotic cells and are a closed sac-like structure composed of two layers of unit membranes. Mitochondria can be divided into the matrix, inner membrane, interstitial space, and outer membrane from the inside to the outside. Among them, the cristae of the mitochondria is a unique structure of the mitochondria, formed by the inwardly protruding folds of the inner mitochondrial membrane. Mitochondria are enriched in locations with high energy demands or locations with strong

metabolic functions. SM mitochondria are distributed under the muscle membrane and between myofibrils (Magalhães et al., 2009, Yu et al., 2016a).

This study aimed to review the effects of EE on the ultrastructure of SM, to provide a reference for further research on the influence of EE on SM.

SM ultrastructure

Ultrastructure of SM under normal conditions

In the longitudinal section of SM under normal conditions, the sarcomere structure can be observed under the electron microscope (Draper and Marshall, 2014). The lines of each band are visible, the structure is complete, and they are arranged regularly. The myofibrils are closely arranged, showing horizontal stripes of light and dark. The size of the mitochondria and nucleus is normal and the structure is complete (Komulainen and Vihko, 1998).

Ultrastructure of SM after a one-time EE

Researchers used animal downhill running sports injury models and found that immediately after performing an EE, the ultrastructure of SM changed: occasionally Z-bands appeared abnormal, myofilament arrangement appeared disordered, and mitochondria occasionally saw vacuole degeneration. The cristae in the mitochondria appeared missing and dissolved; the cell membrane was blurred and dissolved. 12h after an EE, the ultrastructure of SM undergoes further changed: vacuolar degeneration of mitochondria and lack of cristae. The Z-line changes, distortion, and disorder occur locally, and the light and dark bands begin to blur, but the overall situation is still relatively clear. 24 hours after an EE, the ultrastructural damage was more serious: the arrangement of myofibrils was disordered and twisted, the light and dark bands were blurred, and each band and line could not be identified. The Z-line changed obviously, the Z-line was arranged irregularly, it can be seen that the Z-line is zigzag, and the Z-line disappears locally. The nucleus was concentrated and the nuclear membrane was blurred. Mitochondrial vacuolar degeneration and lack of cristae were more common. 48h after an EE, the degree of ultrastructural damage was alleviated compared with 24h after exercise: Z-line was locally misaligned, myofibrils appeared edema, myofibrils were still twisted, mitochondrial outer membrane was partially ruptured, and part of the cristae was missing. There was a phenomenon of vacuole denaturation. 72 hours after an EE, the degree of damage to the ultrastructure of SM was significantly reduced: the arrangement of myofibrils was close to normal, the light and dark bands were clear, the Z line was clear, and the M line could also be distinguished. Some mitochondria also had vacuolar degeneration and a lack of cristae. Mitochondria gradually changed from flocculent changes to myeloid changes (Liu and Li, 2014, Guo et al., 2009). 24h after an EE was the most serious stage of SM ultrastructure damage (Dong et al., 2013). The intensity of EE and the length of exercise time may be the reason for the different degree of SM ultrastructure damage at the same time course.

Ultrastructure of SM after repeated EE

Immediately after repeated EEs, myofibrils appeared irregularly arranged, myofilaments were curled, the Z-line became thinner, and the sarcoplasmic reticulum was enlarged and blurred (Jin et al., 2010b). 24 hours after exercise, necrosis of myofilament can be found, and a large number of mitochondria proliferated near it, which was nearly round and edema. 7 days after exercise, the ultrastructure of SM had been significantly improved, but irregular arrangements of myofibrils, incomplete sarcomeres, crimped myofilaments, thinning of the Z-line, and swelling of the sarcoplasmic reticulum can still be found. After 7 consecutive days of EE training, the degree of damage to the ultrastructure of SMs in different periods was significantly more severe than that of the EE group (Jin et al., 2010a).

The ultrastructural changes of SM immediately after repeated EEs were: a small part of the sarcomere was elongated, the Z-line was clear, and the mitochondrial morphological changes were small except for the decrease in number (Dong et al., 2014). 24h after exercise, the Z-line shifted slightly, the outer mitochondrial membrane was relatively normal, and there was no obvious vacuolation. 48h after exercise, the I-band in the musculature became longer, the Z-line shifted slightly, and individual sarcomeres were blurred. At this time, the mitochondria appeared vacuolization. 7 days after exercise, myofibrils were arranged irregularly, sarcomeres were incomplete, myofilaments were curled, and the Z-line became thinner, but the number of mitochondria returned to normal levels and the structure was completely restored. The degree of damage to the ultrastructure of SM at different time points was lower than that of the EE group, and the mitochondrial structure integrity was higher than that of the EE group (Dong et al., 2013).

Continuous high-intensity EE may lead to the accumulation of SM damage, and the interval between two EEs of one week can make the SM adapt to the EE stimulation, increase the number of mitochondria, strengthen aerobic metabolism, and benefit the bones muscle damage repair.

SM Mitochondria

Distribution and Morphology of SM Mitochondria

The movement of mitochondria in mammalian cells mainly relies on kinesin and dynein (Magalhães et al., 2009). EE leads to changes in the morphological structure of SM, including irregular mitochondria in different sizes (Yu et al., 2016a). The cristae in the mitochondria are significantly reduced, the matrix becomes lighter, and even vacuoles appear. EE also leads to uneven distribution of mitochondria, accumulation of mitochondria under the muscle membrane, and reduction of mitochondria between myofibrils. After a heavy EE, mitochondria were unevenly distributed, mitochondria accumulated under the muscle membrane, and mitochondria were swollen and damaged (Shang et al., 2018). The injury is the most serious 12h after exercise. In a large number of experiments on observing the ultrastructural changes of SM, the above changes have also been observed in mitochondria. After performing an EE, the expression of Miro1/2 protein in SM increased significantly at each time point (Yu et al., 2016a, Yu et al., 2016b). Miro1 reached its highest point 72h after exercise, and Miro2 reached its peak 24h after exercise. Although the expression of KIF5B protein decreased, there was no significant difference. The expression of VDAC1 and Dynlt1 proteins increased, so studies have shown that after a one-time EE, the expression of mitochondrial movement-related proteins increases, which provides power for mitochondrial movement. Combined with the accumulation of mitochondria under the sarcolemma observed under the electron microscope, the mitochondria between the Z discs were reduced. So it is speculated that after EE, the expression of mitochondrial movement-related proteins increased, and mitochondria moved actively, causing the mitochondria between the Z discs to move under the sarcolemma (Yu et al., 2016a, Yu et al., 2016b).

Dynamics of SM mitochondria

Mitochondrial dynamics refers to a dynamic process of mitochondrial division and fusion, which plays an important role in maintaining the transportation of substances and energy in cells (Yu et al., 2014, Liesa and Shirihai, 2013). Under normal circumstances, the fusion and division of mitochondria are in a dynamic equilibrium. If the mitochondrial division is strengthened, mitochondria will become fragmented; if mitochondrial fusion is strengthened, mitochondria will become more networked within the cell (Yu et al., 2014). Changes in mitochondrial dynamics are controlled by related proteins. Proteins related to mitochondrial fusion include Mitofusion 1/2 (Mfn1/2) and optic atrophy 1 (Opa1). Mfn1/2 is located in the outer membrane of mitochondria and mainly regulates the fusion of the outer mitochondrial membrane. Opa1 is located in the inner mitochondrial membrane and is related to the fusion of the inner mitochondrial membrane. The proteins related to mitochondrial division include Dynamin-related protein 1 (Drp1 and fission protein 1 (Fis1). Drp1 and Fis1 participate in the division of the outer mitochondrial membrane (Liu et al., 2016).

After an EE, the expression of SM fusion and split protein showed the opposite dynamic changes (Yu et al., 2014). The expression of Mfn2 and Opa1 decreased immediately after exercise, and the expression of Mfn2 and Opa1 increased from 6h to 24h after exercise. The expression of Mfn1 protein was different from that of Mfn2 and Opa1. Immediately and 6h after exercise, the expression of Drp1 and Fis1 protein increased significantly. Therefore, it is concluded that after a one-time EE, the division of SM mitochondria is strengthened and the fusion is inhibited. About 48h to 72h after exercise, the mitochondrial fusion, and division reach equilibrium again (Yu et al., 2014).

Mitochondrial autophagy

Mitophagy is a selective autophagy, which is the process of identifying, isolating, and degrading damaged or senescent mitochondria (Korolchuk et al., 2017). Mitophagy includes three modes: PINK/Parkin-dependent mitochondrial autophagy, mitochondrial outer membrane receptor-mediated mitochondrial autophagy, and lipid-mediated mitochondrial autophagy.

After an EE, the expression of PINK/Parkin protein in SM showed a trend of first increasing and then decreasing (Shang et al., 2017). The IC3-I/II ratio showed an increasing trend and reached its peak at 12h. Since the conversion of IC3-I to IC3-II can be used to detect autophagy, it is concluded that after a heavy EE, rat SM mitochondria can be Autophagy occurs by activating the PINK/Parkin pathway (Shang et al., 2017).

Mitochondria and Apoptosis

Apoptosis is a morphological concept, which refers to the process in which cells are stimulated by apoptosis signals to maintain the stability of the internal environment and under the regulation of genes to end their lives in the process of development, maturation, and aging (Bran et al., 2008, Bender et al., 2012). The three pathways leading to apoptosis are the endogenous pathway: death factor and its receptor pathway (caspase-8), and the endogenous pathway: mitochondrial pathway (caspase-9) and endoplasmic reticulum pathway (caspase-12).

Mitochondrial pathway induction of apoptosis refers to the increase of mitochondrial membrane permeability after cells receive apoptotic signals, so that pro-apoptotic proteins (CytC, Omi, Smac, etc.) in mitochondria are released into the cytoplasm, activate caspase 9, and then activate caspase 3 to trigger Apoptosis (Siskind, 2005). The study found that after an EE, the expression of CytC protein SM increased, reaching the highest point at 6h after exercise, and returning to normal level at 72h after exercise (Song et al., 2013). It is speculated that CytC was released from mitochondria into the cytoplasm. EE causes CytC in the mitochondria to be released into the cytoplasm, which then initiates the apoptosis program. After EE, the activity of caspase-3 was significantly up-regulated, reaching the highest point at 12h, which proved that EE caused SM cell apoptosis. 12 to 48 hours after exercise, the activity of caspase-9 was higher than the normal level (Song et al., 2013). It is speculated that the caspase-9 pathway is involved in cell apoptosis. After EE, the expression of Omi protein and XIAP both increased, and the combined amount of the two also increased significantly. Therefore, experiments have shown that the pathway of SM cell apoptosis caused by EE includes the mitochondrial pathway. EE leads to an increase of Omi protein expression and promotes Omi. Binding with XIAP then activates the mitochondrial pathway to induce apoptosis. In other studies, it was found that after an EE, the activity of caspase-3 increased, and the expression of the mitochondrial pro-apoptotic protein Smac increased, and there was a positive correlation between the activity level of caspase-3 and the expression level of Smac protein. The activity level of caspase-8 is significantly higher than the normal level from immediately to 24h after exercise, the activity level of caspase-9 is significantly higher than the normal level from 12h to 24h after exercise, and caspase-12 is only high at 12h after exercise. At a normal level, it is speculated that the apoptosis caused by EE may be co-participated by caspase-8, caspase-9, and caspase-12, and mainly depends on the caspase-8 and caspase-9 pathways (Song et al., 2013).

After a heavy EE, the activity of caspase-3 in the SM increased significantly, reaching the highest peak at 12h after exercise, and the activity of caspase-3 returned to normal at 72h, indicating that there is a sequential change in SM cell apoptosis. After three days of exhaustive EE, the apoptosis index SM cells increased immediately after exercise, reaching a peak at 24 hours, and still higher than normal after 48 hours (Song et al., 2013). The test also verified that EE induces apoptosis of SM cells and that there is a phenomenon of sequential changes in apoptosis. Although apoptosis has the characteristics of time sequence, there are differences in the time when the most serious apoptosis occurs. This may be related to the length of exercise designed in the experiment.

Therefore, EE will lead to apoptosis in SM, and there are sequential changes in apoptosis. Exercise intensity and exercise time may be related to the severity of cell apoptosis (Donatella et al., 2000).

Conclusion

In conclusion, this is the first review summarizing the effects of EE on SM ultrastructure and mitochondria. EE may lead to changes in the ultrastructure of SM, including the ambiguity of each line and band in the sarcomere, vacuolar degeneration of mitochondria, swelling of the sarcoplasmic reticulum, and disordered arrangement of myofibrils. Meanwhile, EE also causes to redistribution of SM mitochondria, changes in dynamics, and mitochondrial autophagy. These results provide additional insight into further research on the influence of EE on injuries of SM.

Competing Interests

The authors have no competing interests to declare.

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