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Research Progress on the Impact of MCU on Myocardial Mitochondrial Calcium Metabolism during Exercise

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Abstract: *This article reviews recent research advances regarding the impact of the Mitochondrial Calcium Uniporter (MCU) on myocardial mitochondrial calcium metabolism during exercise, aiming to explore the crucial role of MCU in regulating calcium homeostasis in cardiomyocytes and protecting the myocardium from exercise-induced injury. Through a systematic review and analysis of relevant literature, we summarize the structure and function of MCU, its expression changes during exercise preconditioning and high-intensity exercise, as well as its regulatory mechanisms on myocardial mitochondrial calcium metabolism. Our analysis reveals that MCU exerts a significant influence on myocardial mitochondrial calcium metabolism during exercise, maintaining mitochondrial calcium homeostasis through the regulation of calcium ion transport and thereby protecting the myocardium from damage. Future research should delve deeper into the regulatory mechanisms of MCU and its specific roles in cardiovascular diseases, providing novel targets and strategies for exercise-based cardiac protection and cardiovascular disease treatment.*

Keywords: *Mitochondrial Calcium Uniporter (MCU); Exercise-Induced Myocardial Protection; Mitochondrial Calcium Metabolism*

1. Introduction

Mitochondria, as the core of cellular energy metabolism, synthesize substantial amounts of adenosine triphosphate (ATP) to fuel cellular processes. The regulation of energy metabolism by Ca^{2+} stems from its ability to influence the activity of pyruvate dehydrogenase within the mitochondrial matrix, thereby modulating ATP production. Under normal conditions, the mitochondrial membrane permeabilization transition pore (MPTP) and mitochondrial calcium uniporter (MCU) collaborate to maintain cytosolic Ca^{2+} homeostasis. Elevated Ca^{2+} levels within mitochondria enhance the activity of the $Na^{+}/Ca^{2+}/Li^{+}$-permeable exchanger (NCLX) and promote the opening of MPTP, leading to the release of mitochondrial Ca^{2+}. Excessive accumulation of Ca^{2+} triggers the generation of reactive oxygen species (ROS), which accumulate to induce cellular stress. In response to stress, increased Ca^{2+} concentrations can initiate mitochondrial autophagy, sustaining vital cellular activities. However, persistent stress and excessive Ca^{2+} accumulation ultimately lead to apoptosis.

The molecular characteristics of the calcium uniporter have remained enigmatic for decades until the discovery of the mitochondrial calcium uniporter protein (MCU). Experiments conducted in isolated mitochondria have shown that knocking out MCU nearly completely abolishes calcium uptake, significantly reducing mitochondrial Ca^{2+} accumulation. Subsequent extensive research has consistently demonstrated that silencing MCU significantly reduces mitochondrial Ca^{2+} uptake. Nevertheless, there is a paucity of studies investigating the impact of exercise on MCU, and whether exercise-induced effects on the myocardium are mediated by MCU remains unexplored.

2. Exercise and Calcium Metabolism in Cardiac Mitochondria

2.1 Overview of Calcium Metabolism in Cardiac Mitochondria

Mitochondria contribute to approximately 95% of the energy required for cellular life activities [1]. Additionally, they participate in physiological processes such as apoptosis, cell cycle regulation, and

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cellular development [2]. Research has confirmed that mitochondria can spontaneously absorb and release Ca^{2+} even in an isolated state [3]. However, the question of "what specific roles does Ca^{2+} play in mitochondrial physiological functions" has been explored by the scientific community for nearly half a century [4].

Studies have shown that mitochondrial Ca^{2+} uptake and release play a crucial role in maintaining the dynamic balance of cytosolic Ca^{2+} [5]. The free Ca^{2+} concentration in mitochondria is closely related to changes in mitochondrial energy metabolism and membrane permeability [6-8]. The process of mitochondrial Ca^{2+} uptake and release also modulates intracellular Ca^{2+} signaling [9]. Abnormalities in this process are intimately linked to the development of heart diseases and neurodegenerative disorders [10-12].

Within mitochondria, Ca^{2+} is distributed in the intermembrane space and matrix. The outer mitochondrial membrane exhibits high permeability to Ca^{2+}, resulting in similar Ca^{2+} concentrations in the intermembrane space and cytosol. In the resting state, the Ca^{2+} concentration in the mitochondrial matrix is comparable to that in the cytosol, ranging from 1 to 100 nmol/L [13]. During cellular excitation, cytosolic Ca^{2+} concentrations can reach 2–3 μmol/L, peaking at up to 500 μmol/L [14,15].

Mitochondria possess Ca^{2+} storage capabilities, with Ca^{2+} distributed in both the matrix and intermembrane space. The outer mitochondrial membrane relies primarily on the voltage-dependent anion-selective channel (VDAC) for ion transport. VDAC provides a channel for Ca^{2+} efflux from mitochondria, playing a significant role in maintaining Ca^{2+} homeostasis and regulating cellular activity [16,17]. The transport of Ca^{2+} into the inner mitochondrial membrane is a passive, unidirectional process. Cytosolic Ca^{2+} within a certain concentration range protects mitochondria from calcium overload, with a large-capacity Ca^{2+} buffering system significantly reducing free Ca^{2+} concentrations [18].

Mechanisms of mitochondrial Ca^{2+} uptake include: (1) Ca^{2+} uptake via mitochondrial uniporters [19]; (2) rapid mode of Ca^{2+} uptake (RaM) [20]; (3) Ca^{2+} uptake dependent on mitochondrial ryanodine receptor type 1 [21]; (4) Ca^{2+}/H^{+} exchanger LETM1, mitochondrial Na^{+}/Ca^{2+} exchanger (mNCX) [22], and additional pathways for uptake across the inner membrane.

Furthermore, reactive oxygen species (ROS) attack membrane proteins, leading to ehydrogenation, protein aggregation, crosslinking, and structural changes in membrane proteins, increasing the likelihood of opening permeability transition pores.

2.2 Effect of Exercise on Myocardial Calcium Metabolism

Exhaustive exercise can lead to an increase in Ca^{2+} content in rat mitochondria, as well as increased mitochondrial oxygen free radicals, resulting in mitochondrial calcium overload. This, in turn, causes a decrease in mitochondrial Ca^{2+}-ATPase activity, disruption of mitochondrial membrane integrity, and increased release of free calcium from the mitochondrial matrix, leading to a decrease in its concentration.

Harris et al. [23] demonstrated that exhaustive exercise reduces the content of sulfhydryl groups in mitochondria and elevates Ca^{2+} levels. Activation of phospholipase A2 in the mitochondrial membrane triggers an increase in non-specific permeability, ultimately resulting in increased mitochondrial calcium release. Liu Jianhua et al. [24] conducted a six-week progressive load training on rats, inducing an overtraining state. They observed increased myocardial mitochondrial ROS production, decreased myocardial antioxidant enzyme activity, and excessive oxidative phosphorylation in mitochondria.

Our previous research [25] revealed that high-intensity swimming exercise can induce the opening of mitochondrial permeability transition pores (MPTP) in rat hearts, causing an increase in the outflow of free calcium from the mitochondrial matrix. Zhang Ling et al. [26] found that exhaustive exercise triggers significant elevations in lipid peroxidation levels and phospholipase A activity in rat myocardial mitochondria, accompanied by decreases in superoxide dismutase (SOD) activity and free Ca^{2+} concentration. Furthermore, numerous new calcium channels emerged on the mitochondrial membrane, enhancing the release of free calcium from mitochondria.

3. Mitochondrial Calcium Uniporter (MCU)

3.1 Overview of Mitochondrial Calcium Uniporter (MCU)

Human Mitochondrial Calcium Uptake 1 (MICU1) exists in three splice variants, with the

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full-length MICU1 having a molecular weight of 54 kDa and located on the mitochondrial inner membrane. It contains a primary transmembrane helix and two calcium-binding domains (EF hands). It is speculated that MICU1 itself cannot form a pore but regulates MCU [27]. Baughman et al. [28] and Stefani et al. [29] discovered MCU proteins in the mitochondrial inner membrane that interact with MICU1 to mediate mitochondrial Ca^{2+} uptake.

The MCU monomer is 40 kDa in size and contains two transmembrane helices. The interface between the helices is rich in acidic amino acids essential for calcium ion transport. Native PAGE results suggest that the monomeric protein functions in a highly oligomeric form for calcium ion uptake. Subsequent studies found that knocking out MCU in isolated mitochondria almost completely inhibited calcium uptake, and mitochondrial Ca^{2+} uptake was also significantly reduced in intact cells. Patch-clamp measurements showed that knocking out MCU blocked Ru360-sensitive Ca^{2+} currents. Meanwhile, transient spontaneous changes in mitochondrial Ca^{2+} concentration were observed, while cytosolic Ca^{2+} concentration remained unchanged, indicating the presence of an autonomous Ca^{2+} signaling mechanism in mitochondria. MCU is thus presumed to be the protein responsible for regulating Ca^{2+} uptake. MCU, together with regulatory molecules such as MICU1 and MICU2, forms a complex to regulate Ca^{2+} uptake [30].

Silencing MCU significantly reduces mitochondrial Ca^{2+} uptake, but Holmstrom [31] and Pan [32] et al. found that substantial Ca^{2+} could still be detected in mitochondria of MCU-silenced mouse cells, indicating that MCU is not the sole mechanism for mitochondrial Ca^{2+} uptake [33].

3.2 Structure of MCU

In 2011, Baughman et al. [23] examined MCU proteins related to MICU1 function, specifically the CCDC109A protein. Using proteomics analysis, they found that MCU has two predicted transmembrane domains, structurally similar to but distinct from MICU1. Ru360 was identified as an effective inhibitor of MCU, suggesting its involvement in mitochondrial calcium ion uptake. Since then, MCU has garnered increasing attention, particularly in cardiomyocytes, neurons, and hepatocytes [23].

The MCU nuclear gene is located on chromosome 10 and encodes a 40 kDa protein that converts to a mature 35 kDa form during mitochondrial uptake. MCU contains two transmembrane helices, with both the N- and C-terminal domains spanning the membrane into the mitochondrial matrix [34]. Studies have shown that MCU oligomers can form functional, unidirectional transport channels, with eight helices arranged along a hypothetical channel region. Charged amino acid residues line adjacent regions of the channel, creating a negative potential that favors cation passage [35].

3.3 Function of MCU

The primary function of MCU (mitochondrial calcium uniporter) is to transport Ca^{2+} from the cytosol into the mitochondrial matrix and control the rate of this transport, which is crucial for intracellular Ca^{2+} signaling, calcium homeostasis, mitochondrial energy metabolism, and apoptosis. Ca^{2+} regulates mitochondrial oxidative phosphorylation via MCU, and its phosphorylation accelerates cellular Ca^{2+} uptake, facilitating ATP production. However, excessive phosphorylation may lead to mitochondrial calcium overload, excessive ROS generation, potentially damaging mitochondria and inducing apoptosis [36,37].

The driving force for MCU-mediated Ca^{2+} uptake primarily relies on the concentration gradient and the electrical potential difference across the mitochondrial membrane. Mitochondria, as the core of cellular energy metabolism, are where most ATP is generated. Under physiological conditions, Ca^{2+} serves as a key regulator of mitochondrial function, with mitochondrial Ca^{2+} uptake buffering concentrations both inside and outside the organelle, stimulating ATP synthesis at multiple levels within the cell [38]. During oxidative stress, mitochondrial homeostasis is disrupted, and Ca^{2+} overload can damage cells, emphasizing the necessity for precise regulation of Ca^{2+} in mitochondria.

Intracellular ROS are primarily generated by mitochondria and are also their target. Under normal physiological conditions, low levels of ROS maintain cellular function, regulating metabolism and signal transduction; excessive ROS production, however, leads to oxidative stress, damaging cell function and predisposing to cardiovascular diseases [39]. Studies by Giacomello [40] and Pivovarova [41] found that mitochondrial stress can cause excessive ROS production and calcium overload, subsequently stimulating the opening of mitochondrial MPTP (mitochondrial permeability transition pore), damaging mitochondria, releasing apoptotic factors, and ultimately leading to apoptosis.

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Forstermann et al. [39] revealed that mitochondrial dysfunction contributes to the development of diabetic vascular complications, where excessive ROS generation and oxidative stress are key mechanisms of vascular dysfunction [39].

3.4 Effect of Exercise on MCU

While studies on the direct effect of exercise on MCU are limited, research on the impact of exercise on MPTP pores is more established. Studies have shown that exhaustive exercise can damage mitochondria, impairing their function. Short-term mitochondrial structural and functional decline has been observed. Biochimica et al. [42] reported that MPTP is crucial in the apoptotic process. Using confocal microscopy to observe MPTP during TNF-α-induced hepatocyte apoptosis, they found that MPTP membrane permeability changes, leading to decreased mitochondrial membrane potential, ATP depletion, oxidative phosphorylation decoupling, mitochondrial swelling, outer membrane rupture, release of apoptotic factors, and ultimately apoptosis or necrosis. These mitochondrial changes are also major factors contributing to exercise-induced fatigue [43].

Hu Zhigang et al. [44] studied the effects of exhaustive exercise and aerobic training on MPTP pores, finding that after exhaustive exercise, mitochondrial membrane potential decreased, and mitochondrial Ca^{2+} was released in large quantities. In contrast, after aerobic training, mitochondrial membrane potential and Ca^{2+} loss were significantly reduced, mitigating mitochondrial damage. Clinical trials by Guardian [45] showed that using Na+/H+ exchange inhibitors in CABG (coronary artery bypass grafting) patients significantly improved their prognosis. Reduced calcium overload also reduced mitochondrial damage, blocking a series of pathological reactions.

4. Conclusion

This study reviews the recent research progress on the effects of MCU on myocardial mitochondrial calcium metabolism during exercise, revealing the crucial role of MCU in regulating myocardial cell calcium homeostasis and protecting the myocardium from exercise-induced damage. MCU achieves precise regulation of mitochondrial calcium metabolism through its unique structure and function, maintaining intracellular calcium homeostasis and energy metabolism balance. Exercise preconditioning can reduce MCU activity and alleviate mitochondrial calcium overload, thereby protecting the myocardium from exercise-induced damage. In contrast, high-intensity exercise may increase MCU expression and activity, leading to mitochondrial calcium overload and myocardial damage. Future research should further explore the regulatory mechanisms of MCU and its specific roles in cardiovascular diseases, providing new targets and strategies for exercise-induced cardiac protection and cardiovascular disease treatment.

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