Life Sciences 224 (2019) 157–168

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Trimetazidine combined with exercise improves exercise capacity and anti-fatal stress ability through enhancing mitochondrial quality control

Murong Xie[1], Lingjun Jiang[1], Yaoshan Dun, Wenliang Zhang, Suixin Liu*

Cardiac Rehabilitation Center, Department of Rehabilitation, National Clinical Research Center for Geriatric Disorders, Xiangya Hospital of Central South University, Changsha 410008, China

ARTICLE INFO

Keywords:
Trimetazidine
Aerobic exercise
Exercise capacity
Anti-fatal stress ability
Skeletal muscle damage
Mitochondrial quality control

ABSTRACT

Aims: To explore the effects of trimetazidine combined with exercise on EC and anti-fatal stress ability, and illustrate the underlying mechanism.

Methods: C57BL/6 mice were randomly assigned to four groups ($n = 11$ in each group): the control, exercise, trimetazidine and trimetazidine + exercise (TE) groups. Mice were accordingly given saline (ig), Aerobic exercise (AE), trimetazidine (ig), or a combination of trimetazidine (ig) and AE for five weeks. After the intervention, each group was randomly subdivided into rest and exhaustive exercise (EE) subgroups. The mice in the control-EE and TE-EE subgroups underwent fatal stress experiments. EC and anti-fatal stress ability were assessed respectively. Mitochondrial quality control (MQC) in skeletal muscle were measured at the protein level and the organelle level.

Key findings: A significantly increased exhaustive swimming time was observed in exercise (39.10 ± 12.58 min *vs* 14.18 ± 4.37 min), trimetazidine (33.73 ± 8.45 min *vs* 14.18 ± 4.37 min) and TE groups (73.78 ± 18.95 min *vs* 14.18 ± 4.37 min) compared with that in the control group, and a synergistic effect was detected ($P < 0.05$). Fatal stress experiments successfully induced skeletal muscle damage, including increased creatine kinase activity, myofibrosis, and impaired antioxidative enzyme system, all those were significantly alleviated by trimetazidine supplementation combined with AE precondition ($P < 0.05$). Meanwhile, AE and trimetazidine alone or combined, significantly enhanced the MQC in normal mice by activating mitochondrial biogenesis, dynamics and mitophagy, and that in mice underwent fatal stress stimulus ($P < 0.05$).

Significance: This study for the first time found that trimetazidine and AE have synergistic effects on improving EC. Moreover, the combination of both interventions enhances anti-fatal stress ability. Enhancing MQC may be a key mechanism of AE combined with trimetazidine that improves EC and anti-fatal stress ability.

1. Introduction

Cardiovascular disease (CVD) remains the leading cause of death worldwide, and its prevalence continues to increase [1]. Recently, studies showed that the incidence of CVD in young people (18–50 years old) has stabilized or increased over the past decades, while declined in adults over 50 years old. Those observations suggest a trend toward a younger population of patients with CVD [2]. Moreover, it is demonstrated that exercise capacity (EC), the fifth vital sign, is an independent predictor of cardiovascular disease, diabetes, and all-cause mortality [3,4]. Patients with higher ECs have lower death rates after stroke [5,6] and cancer [7]. In addition, the survival rate and prognosis in patients with acute myocardial infarction [8], burn [9], stroke [10,11] or other fatal stress states [12] are poor, despite the fact that they may have been receiving advanced medical treatment. Thus, improving the anti-fatal stress ability is an effective measure for reducing the occurrence of fatal adverse cardiovascular events [13,14], and enhancing their prognosis. Accordingly, the development of methods for improving EC and anti-fatal stress capacity and the discovery of the underlying mechanisms are of great clinical significance.

Skeletal muscle is an important target organ for research on EC. However, studies on the relationship between skeletal muscle stress and cardiovascular events are rare. Recently, it is revealed that skeletal muscle produces hundreds of different kinds of cytokines and peptides that affect the function of the cardiovascular system, immune system, skeletal muscle itself and other system [15]. Skeletal muscle dysfunction may be one of the pathological mechanisms of heart failure and other cardiovascular diseases [16]. Therefore, skeletal muscle is not

* Corresponding author.
E-mail address: heartsuixin@126.com (S. Liu).
[1] The authors contributed equally to this work.

https://doi.org/10.1016/j.lfs.2019.03.027
Received 8 January 2019; Received in revised form 4 March 2019; Accepted 10 March 2019
Available online 12 March 2019

only an important part of research on EC but also an essential target organ for studying stress injury response and the related mechanisms. Mitochondria, a highly dynamic organelle, are the centre of aerobic metabolism, and their function is an important indicator of EC [17,18] and anti-fatal stress capacity [19]. Mitochondrial quality control (MQC) is an effective guarantee of normal mitochondrial function [20,21]. It has been reported that MQC is a potential therapeutic target for addressing mitochondrial dysfunction as well as a treatment method for neurodevelopmental disorders and neurodegenerative diseases [22,23]. Therefore, the present study focuses on mitochondrial function and MQC as the target mechanism.

At present, improving EC or anti-fatal stress capacity mainly includes pharmaceutical and non-pharmaceutical methods. Exercise, an essential non-pharmacological treatment, significantly improved the EC of patients with coronary heart disease [24,25], and anti-fatal stress capacity [26] in mice. Moreover, our previous study showed that AE improved EC and ameliorated exhaustive exercise-induced muscle damage and that these effects were closely related to MQC [26]. Trimetazidine (TMZ) has been widely used in patients with coronary artery disease and metabolic disorders [27]. Meta-analysis [27] and randomized longitudinal studies [28] suggested that TMZ improved EC in patients with ischaemic heart disease, existing clinical and basic research on improving EC and the anti-fatal stress ability of TMZ has mainly focused on the myocardium [29–31]. Recently, Molinari F et al. [32] revealed that TMZ enhanced the fast-to-slow myofibre phenotype shift, peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC1-α) upregulation, oxidative metabolism, and mitochondrial biogenesis in C26-bearing mice. Gatta L et al. [33] also showed that at the cellular level, TMZ upregulated the AMP-activated protein kinase (AMPK)/PGC1α signalling pathway to promote mitochondrial biogenesis and activated autophagy in skeletal muscle. However, studies examining the mechanisms of TMZ-induced improvements EC in skeletal muscle are limited, nor the synergistic effect of TMZ combined with AE on EC and its ability to relieve stress injury.

In this study, we observed whether TMZ and AE have a synergistic effect on the improvement of EC *via* MQC in skeletal muscle. To further verify the effect of TMZ combined with AE on survival rate and prognosis after fatal stress, we evaluated whether TMZ supplementation combined with AE could alleviate fatal stress-induced damage and enhance anti-fatal stress ability, and elucidated its underlying mechanisms.

2. Materials and methods

2.1. Animals and study design

Forty-four healthy male C57BL/6 mice (8 weeks old), weighing 22.66 ± 2 g, were used. After adaptive feeding for one week, the mice were randomly assigned to 4 groups (n = 11 in each group)—control, TMZ, exercise and TMZ + exercise (TE) groups—using a randomized block design according to the animals' weights. During the experimental period, the mice were housed at 22 ± 2 °C and maintained at a relative humidity of 45–55% on a 12/12 h light–dark cycle with free access to water and food. The mice were reared in a clean environment, and the bedding and feeding boxes were replaced every other day. None of the mice had any swimming experience before the experiment, and this experiment was started after 1 week of adaptive feeding. The mice in each group were accordingly given saline (ig, 0.1 ml/10 g body weight), TMZ (Schweageer Pharmaceuticals, ig, 10 mg/kg body weight per day) [31], AE training or TMZ administration combined with AE training for 5 weeks. After the intervention, each group was randomly subdivided into rest and exhaustive exercise (EE) subgroups. The mice in the control-EE and TE-EE subgroups underwent fatal stress experiments (see below for details of the fatal stress model) prior to sacrifice after approximately 12 h. During the experiment, one mouse in the control group died of asphyxiation caused by the gavage procedure, and therefore it was excluded.

2.2. Experimental protocol

2.2.1. Animal aerobic exercise training protocol

Based on training methods reported in the literature [34], our team has explored a set of reformative exercise programmes after years of practice [26], and details are as follows: mice in the exercise and TE groups underwent non-weight-bearing moderate-intensity swim training in a Morris water maze pool (type number XR-XM101-R, 60 cm high, 120 cm in diameter) with a water depth of 30 cm maintained at 30 ± 2 °C. The mice initially swam freely for 10 min on the first day. The swimming time was then gradually increased to 60 min/day by adding 10 min each day. The swim training consisted of forced swimming with the mouse tethered to a stick with string for 60 min, 6 days/week for 4 weeks.

2.3. Fatal stress experiment process

The mice in the control-EE and TE-EE subgroups were subjected to the fatal stress model, which involved an EE test. The specific steps of the EE test [35] with some improvements are as follows: the mouse had a 5% body-weight load attached to its tail and swam until exhaustion, which was defined as the point at which the mouth and nose of the mouse was completely submerged in water for up to 7 s. The depth of the swimming pool was no < 30 cm, and the water temperature was maintained at 32 ± 2 °C. All mice underwent a pre-experiment.

2.4. Assessment of EC

The mice in the EE subgroups (n = 5 mice from the control-EE subgroup and n = 6 from the other EE subgroups) underwent the EE test at the end of the experiment, and the exhaustive swimming time (the duration of forced weight-loaded swimming until the mouth and nose of the mouse were completely submerged in water for up to 7 s) was recorded as the measurement of EC.

2.5. Tissue processing

Mice were anaesthetized with 5% chloral hydrate (0.1 ml/10 g weight) *via* an intraperitoneal injection and then sacrificed after a blood sample was taken. Once a surgical plane of anaesthesia was reached, a thoracotomy was performed, and blood was obtained *via* removal the eyeball. Plasma was separated and stored at −20 °C until required. The quadriceps, gastrocnemius and extensor digitorum longus of each mouse were excised.

2.6. Isolation of mitochondria from skeletal muscle

The mitochondria from skeletal muscle (quadriceps femoris) of the mice were isolated using differential centrifugation according to established protocols (C3606, Tissue mitochondrial isolation kit, Beyotime, China).

2.7. Ultramicrostructure of skeletal muscle under electron microscopy

To further visualize MQC at the organelle level, the mitochondria and autophagosome number in the quadriceps femoris (n = 2 muscles per group) were observed under electron microscopy (EM, Tecnai G2 Spirit, FEI, USA).

2.8. Measurement of anti-fatal stress ability

To evaluate anti-fatal stress ability, plasma creatine kinase concentrations, skeletal muscle ultrastructure under EM, mitochondrial oxidative stress and the antioxidative enzyme system were assessed. As

creatine kinase (CK) is a marker of damage in CK-rich tissue such as skeletal muscle [36], the CK in serum samples from mice was assayed using an assay creatine kinase kit (A032, Nanjing Jiancheng Bioengineering Institute, China). In addition, the morphology of muscle myofibres and mitochondria in skeletal muscle were observed under EM to further evaluate EE-induced skeletal muscle injury.

2.9. Assessment of mitochondrial oxidative stress and the antioxidative enzyme system in skeletal muscle

To assess mitochondrial oxidative stress, levels of malondialdehyde (MDA), which indicate the degree of lipid peroxidation, were measured by a thiobarbituric acid test (A003-1, Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. To assess the antioxidative enzyme system, superoxide dismutase (SOD) activities and glutathione peroxidase (GSH-Px) content in the gastrocnemius muscle as well as the mitochondrial SOD (Mit-SOD) activities in the quadriceps were detected biochemically. SOD activity was measured by the xanthine oxidase method with a SOD Assay Kit (A001-1, Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. The GSH-Px content was measured by reading the absorbance at 412 nm using colorimetric determination (A005, Nanjing Jiancheng Bioengineering Institute, China) and expressed as U/mg tissue protein.

2.10. Mitochondrial function assay

To determine whether TMZ and TMZ combined with AE improved mitochondrial function in skeletal muscle, we analysed the expression of CS. CS, the first and rate-limiting enzyme of the tricarboxylic acid cycle, plays a key role in regulating energy production during mitochondrial respiration [37].

2.11. Assessment of the MQC in skeletal muscle

To determine whether TMZ and TMZ combined with AE improved MQC in mouse skeletal muscle (quadriceps femoris), we analysed the expression of mitochondrial biogenesis, dynamics and mitophagy in the basal state and fatal stress state. Mitofusin 1 (MFN1) and dynamin-related protein 1 (DRP1), direct executors of mitochondrial dynamics [21], and the AMPK/PGC-1α signalling pathway, which reflects mitochondrial biogenesis, were analysed by western blotting. The mitochondrial autophagic protein BNIP3 (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3), microtubule-associated protein 1 light chain 3 (LC3)-II and p62 (SQSTM1, sequestosome) are all directly involved in the formation of mitophagy [38–41]. Therefore, according to the “Guidelines for the use and interpretation of assays for monitoring autophagy”, BNIP3, LC3-II, LC3-I and p62 were all detected by western blotting, and the LC3-II/LC3I ratio was calculated to evaluate mitophagy activity in skeletal muscle. EM was used to further confirm the number of mitochondria and autophagosomes at the organelle level.

2.12. Protein assay

For protein content analysis, skeletal muscle samples were homogenized and analysed by western blotting. The following antibodies were used: CS (16131-1-AP, rabbit, 1:1000), Phosphorylation of AMP-activated protein kinase (pAMPK) (ab131357, rabbit, 1:500) and BNIP3 (ab109362, rabbit, 1:1000) from Abcam (England); PGC-1α (sc-13067, rabbit, 1:200) from Santa Cruz (USA); MFN1 (13798-1-AP, rabbit, 1:500), DRP1 (12957-1-AP, rabbit, 1:500), LC3-II/I (14600-1-AP, rabbit, 1:500), and p62 (18420-1-AP, rabbit, 1:1000) from Proteintech (USA); and GAPDH (AP0063, rabbit, 1:5000) from Bioworld (USA). Membranes were analysed and quantified using a Quantity One Imaging System (BIO-RAS, USA). Protein expression was normalized to that of GAPDH.

2.13. Statistical analyses

All experimental data were input into SPSS 19.0 for statistical analysis and expressed as the mean and standard deviation ($\bar{x} \pm SD$). Statistical significance was determined using one-way analysis of variance followed by the variance homogeneity test. $P < 0.05$ was considered to indicate significance.

3. Results

3.1. Effects of TMZ and AE, alone or combined, on EC and MQC in skeletal muscle of mice

3.1.1. TMZ and AE, alone or combined, improved EC

The exhaustive swimming time was recorded as a measure of EC as described previously. The exhaustive swimming time of the TMZ group (33.73 ± 8. 45 min *vs* 14.18 ± 4.37 min, $P < 0.05$), exercise group (39.10 ± 12.58 min *vs* 14.18 ± 4.37 min, $P < 0.01$) and TE group (73.78 ± 18.95 min *vs* 14.18 ± 4.37 min, $P < 0.01$) were all significantly prolonged compared with that of the control group. Furthermore, compared with the TMZ and exercise groups, the TE group had a significantly lengthened exhaustive swimming time (Fig. 1).

3.1.2. TMZ and AE, alone or combined, enhanced mitochondrial function

The expression of skeletal muscle CS, an indicator of mitochondrial function [37], was greatly increased in mice with interventions of TMZ supplementation and AE, alone or combined compared with mice without interventions ($P < 0.01$). Moreover, compared with the TMZ and exercise groups, the expression of CS in the TE group significantly increased ($P < 0.01$) (Fig. 2A–B).

3.1.3. TMZ and AE, alone or combined, enhanced MQC of skeletal muscle

Mitochondrial biogenesis, dynamics and mitophagy are three important processes that regulate MQC [20,21]. The expression levels of pAMPK, PGC-1α, MFN1, DRP1 and BNIP3 from skeletal muscle in the basal state were all significantly upregulated, as well as the LC3-II/LC3-I ratio, while P62 was significantly downregulated in the exercise and TE groups compared with those in the control group ($P < 0.05$), and TMZ had the same effect in skeletal muscle, as shown in Figs. 3A–C, 4A–C and 5A–D. Moreover, mice in the TE group presented significantly higher expressions of pAMPK, PGC-1α, MFN1, DRP1, BNIP3 and the LC3-II/LC3-I ratio in skeletal muscle, and lower expression of P62 than mice in the TMZ and AE groups ($P < 0.05$). In addition, the number of mitochondria and autophagosomes between the nucleus and myofibrils were considerably increased in the TMZ, exercise, and TE groups under

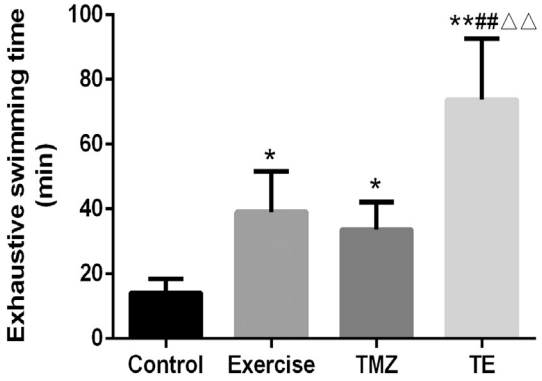

Fig. 1. TMZ and AE, alone or combined, prolonged the exhaustive swimming time in mice. The exhaustive swimming time was measured by the EE test. Values are reported as the *mean* ± *SD* ($n = 5$ mice from the control group and $n = 6$ from other groups). $^{\star}P < 0.05$, $^{\star\star}P < 0.01$ *versus control*; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *versus exercise*; $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$, *versus TMZ*.

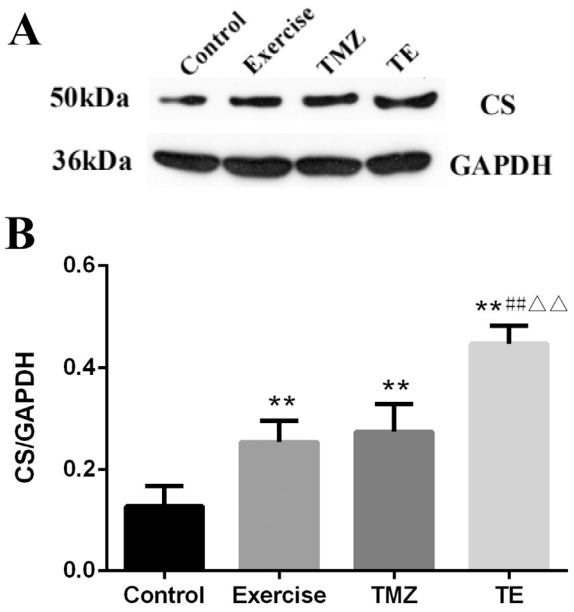

Fig. 2. Effects of TMZ and AE, alone or combined, on mitochondrial function. The expression of CS (B) in skeletal muscle was significantly increased in the TMZ, exercise and TE groups. Values are reported as the *mean* ± *SD* ($n = 3$ muscles per group). $^{\star}P < 0.05$, $^{\star\star}P < 0.01$ *versus control*; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *versus exercise*; $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$, *versus TMZ*. Bands from the western blotting analysis (A).

EM (Figs. 3D, 5E).

3.2. Effects of TMZ combined with AE on anti-fatal stress ability and MQC in skeletal muscle after EE

3.2.1. EE-induced skeletal muscle damage was ameliorated by TMZ combined with AE

The serum CK activity of mice that underwent EE-induced fatal stress in the control-EE subgroup was 2.64-fold higher than that in the mice in the control group ($P < 0.01$). This effect was significantly attenuated in the TE-EE subgroup preconditioned with TMZ supplementation combined with AE ($P < 0.01$) (Fig. 6A). In addition, EE resulted in severe skeletal muscle fibre damage, displaying myofibre necrosis, disordered sarcoma, blurred or missing Z-lines and H bands in myofibres, and moderate-to-severe mitochondrial oedema under EM. TMZ and AE jointly and significantly mitigated the skeletal muscle damage mentioned above induced by EE (Fig. 6B).

EE exerted a positive impact on mitochondrial oxidative stress and inhibitory effects on the antioxidative enzyme system, which were reversed by TMZ combined with AE.

EE significantly increased the level of skeletal muscle MDA, an indicator of mitochondrial oxidative stress, by 37.8% ($P < 0.01$) and decreased the activities of Mit-SOD, SOD and GSH-Px, indicators of the antioxidative enzyme system, by 89.5%, 16.3% and 45.8%, respectively ($P < 0.05$). TMZ combined with AE notably reversed the changes resulting from EE, and the level of MDA in skeletal muscle dramatically decreased, while the activities of Mit-SOD, SOD and GSH-Px significantly increased compared with those of mice in the control-EE subgroup ($P < 0.01$) (Fig. 7A–D).

3.2.2. TMZ combined with AE enhanced the MQC in skeletal muscle of mice underwent EE

The expression levels of pAMPK, PGC-1α, MFN1, DRP1, and BNIP3 and the LC3-II/LC3-I ratios were significantly increased, while the expression of P62 was obviously decreased in the TE-EE group compared with those in the control-EE group ($P < 0.05$), as shown in Figs. 8A–E and 9A–E. At the same time, the number of autophagosomes between the nucleus and myofibrils was considerably higher in mice in the TE-EE subgroup than in those in the control-EE subgroup (Fig. 9E).

4. Discussion

4.1. TMZ and TMZ combined with AE improved EC through enhancing the MQC in skeletal muscle

We confirmed that TMZ improved EC, which was represented by an increase in exhaustive swimming time in mice treated with TMZ intervention. The EE test is one method for assessing anti-fatigue fitness. Because of the mouse survival instinct, this test can truly measure the maximal EC of mice and is commonly used to evaluate EC [35]. The duration of exhaustive swimming time was used as an indicator of EC. The longer the exhaustive swimming time was, the stronger the EC. Therefore, our results suggest that TMZ significantly improves the EC of C57BL/6 mice, which is consistent with the findings of previous studies [32,42]. However, the underlying mechanism of TMZ-associated EC needs to be elucidated to improve the prognosis of patients with cerebrovascular disease and cardiopathy.

In this study, increased CS activity and MQC were observed in skeletal muscles from mice with TMZ supplementation. MQC is an effective guarantee of the normal operation of mitochondrial function [20,43]. The MQC process occurs at both the molecular and organelle levels. Previous studies on MQC focused on the level of mitochondrial protein quality control. In recent years, with the emergence of mitophagy research, an increasing number of studies have concentrated on the organelle level of MQC, which mainly refers to the interaction between mitochondrial biogenesis, dynamics and mitophagy. These processes are intensively discussed to ensure the MQC [20,22,43,44] and maintain intracellular mitochondrial homeostasis [22,44]. Accordingly, the significantly improved balance between mitochondrial biogenesis, dynamics and mitophagy in the mice treated with TMZ in the present study contributes to the observed increase in EC.

Moreover, studies have shown that AE could upregulate the AMPK/PGC-1α signalling pathway and promote skeletal muscle energy metabolism, improving EC [26,45]. Impaired EC was presented in skeletal muscle-specific AMPK$\alpha_1\alpha_2$ double knockout mice [46], muscle-specific AMPK$\beta_1\beta_2$ knockout mice [47] or PGC-1α knockout mice [48]. Therefore, our results suggested that TMZ, similar to AE training [49], is an important way to improve EC by upregulating mitochondrial biogenesis of skeletal muscle, as shown in Fig. 3A–C. In addition, this study for the first time found that TMZ promoted mitochondrial dynamics and activated mitophagy in skeletal muscle in the basal state *in vivo*. The quality of the mitochondrial fusion-fission cycle is the core link in maintaining the mitochondrial life cycle. During the process of mitochondrial dynamics [21], mitochondrial fission stimulating factors, such as DRP1, “distribute” mitochondria into two daughter parts: the inactivated and activated mitochondria [50], through continuous mitochondrial fusion and fission, the inactivated mitochondria are progressively isolated from the mitochondrial network, which is then enveloped by mitophagy for the degradation and recycling of damaged mitochondria. The activated mitochondria continuously enter the new fusion and fission network cycle under the regulation of MFN1/2 [51]. Mutation or inhibition of DRP1 was demonstrated to prevent the activation of mitophagy [52,53], indicating that the disruption of mitochondrial fission disabled dysfunctional mitochondria from being cleared by mitophagy. Interestingly, TMZ ameliorated mitochondrial function [31], mitochondrial biogenesis, and autophagy [29] in skeletal muscle at the cellular level *ex-vivo*. In this study, TMZ simultaneously upregulated the expression of MFN1 and DRP1 to promote mitochondrial dynamics and activated mitophagy-active protein BNIP3 and LC3-II to increase mitophagy in skeletal muscle in the basal state. Meanwhile, the number of mitochondria and autophagosomes in skeletal muscle under EM and CS activity in mice treated with TMZ also significantly increased. Therefore, the present study for the first time

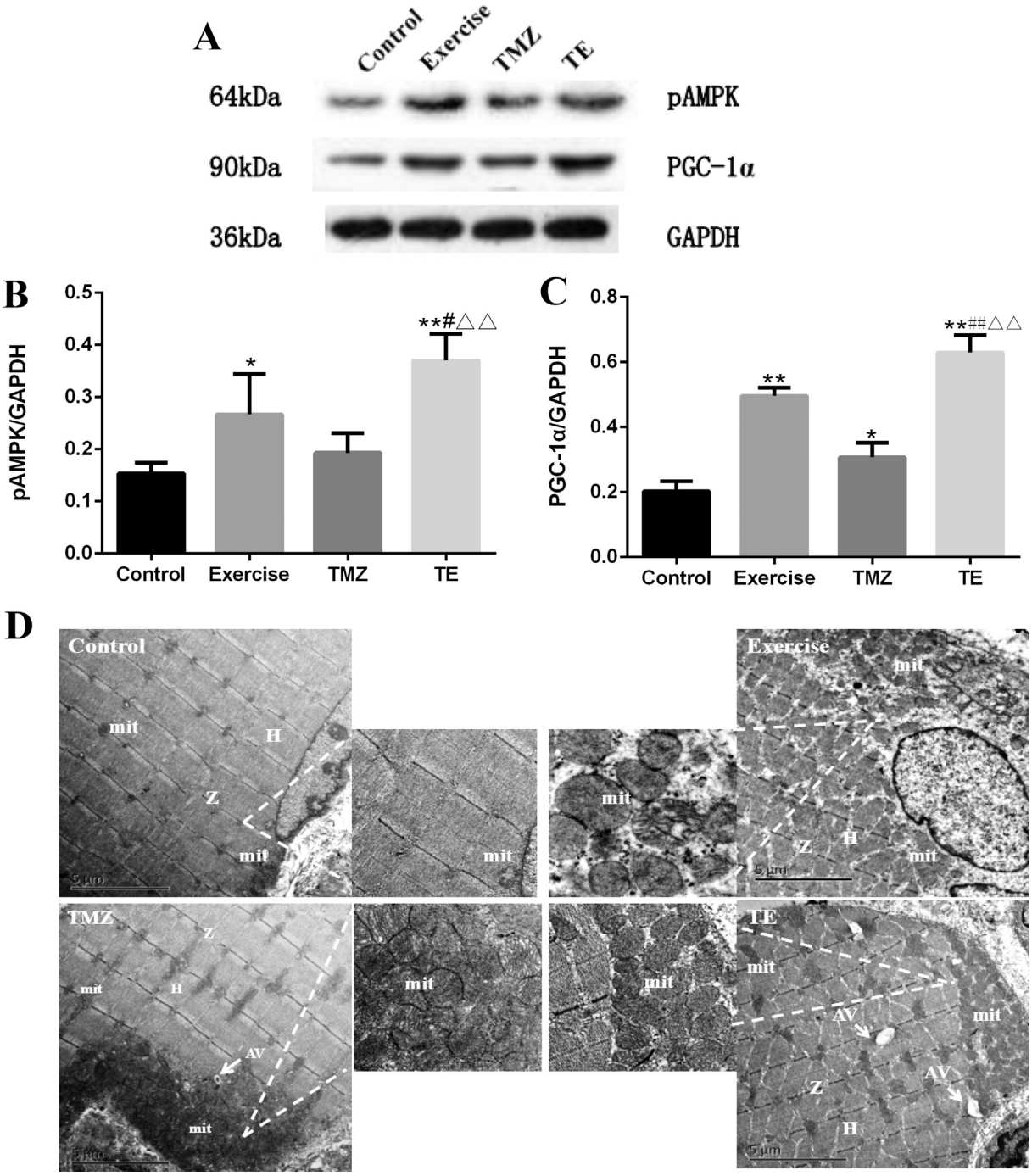

Fig. 3. TMZ and AE, alone or combined, improved mitochondrial biogenesis in the basal state, Representative pAMPK (B), PGC-1α (C) and EM micrographs (D) from skeletal muscle in mice in the basal state. EM shows that the mitochondria number (D) in skeletal muscle was significantly increased in the TMZ, exercise and TE groups. mit: mitochondria, AV: autophagic vacuole, Z: Z line, H: H band. Bars: A: 5 μm; inset, 2 μm. Values are reported as the *mean* ± *SD* (n = 3 muscles per group). $^{\star}P < 0.05$, $^{\star\star}P < 0.01$ *versus control*; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *versus exercise*; $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$, *versus TMZ*. Bands from the western blotting analysis (A).

revealed that TMZ improved MQC in skeletal muscle *in vivo*. These results proved that TMZ contributes to the positive effects on maintenance of MQC and then improves mitochondrial function, which plays a significant role in improving EC.

In addition, TMZ combined with AE had a synergistic effect on improving EC, which was indicated by a significant increase in exhaustive swimming time in the TE group that was 1.4-fold longer than that in the exercise group and 2.0-fold longer than that in the TMZ group. This finding is consistent with previous clinical [54] or basic [55] studies. Our results indicate that MQC contributes to this synergistic effect for the following reasons: First, the combination of TMZ and AE further promotes mitochondrial biogenesis in skeletal muscle by increasing the mitochondrial number and mass entering the mitochondrial life cycle. And the increased pAMPK and PGC-1α in skeletal muscle contributes to the mitochondrial biogenesis, which involves two pathways: first, promoting the phosphorylation of AMPK and then the expression of its downstream target, PGC-1α; second, directly increasing PGC-1α expression. This finding has been confirmed in previous studies [56,57] showing that the expression of PGC-1α was reduced by only 40% in AMPK knockout mice, which means that the expression of PGC-1α is gradually regulated by AMPK [56,57] and the native promoter, PGC-1α. Second, TMZ combined with AE further

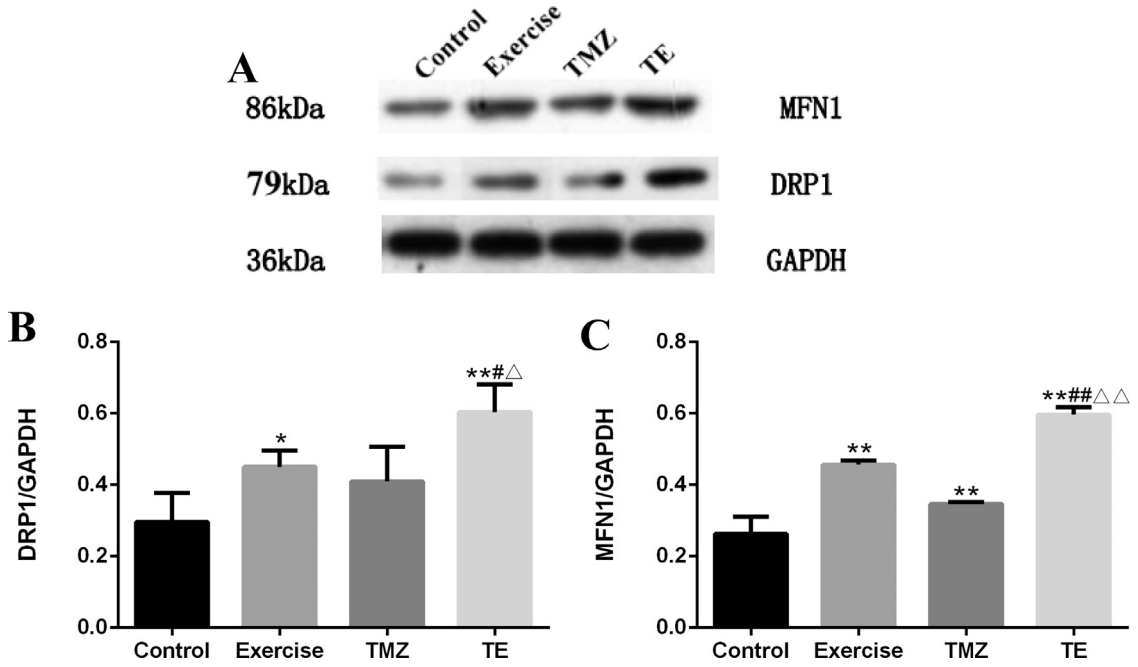

Fig. 4. TMZ and AE, alone or combined, promoted mitochondrial dynamics in the basal state, The expression levels of MFN1 (B), and DRP1 (C) from skeletal muscle in mice. Values are reported as the *mean* ± *SD* (n = 3 muscles per group). $^{\star}P < 0.05$, $^{\star\star}P < 0.01$ *versus Control*; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *versus exercise*; $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$, *versus TMZ*. Bands from the western blotting analysis (A).

activates mitophagy in skeletal muscle, promoting the elimination of dysfunctional mitochondria and supporting skeletal muscle plasticity in response to AE. Finally, TMZ combined with AE further promotes mitochondrial dynamics presented as an upregulation of MFN1 and DRP1, which enhances the regulatory efficiency of the mitochondrial fission-fusion cycle network, and maintains mitochondrial function. Additionally, the increase in CS activity and number of mitochondria and autophagosomes in skeletal muscle under EM further confirmed that TMZ combined with AE could maintain mitochondrial function. Together, Improving MQC in skeletal muscle is a vital factor contributing to the synergistic effect of TMZ combined with AE on enhancing EC.

4.2. TMZ combined with AE alleviated fatal stress-induced skeletal muscle damage and enhanced anti-fatal stress ability through improving MQC

We confirmed that TMZ combined with AE alleviated fatal stress-induced skeletal muscle damage and enhanced anti-fatal stress ability. In this study, skeletal muscle injuries and antioxidative enzyme system damage induced by fatal stressor-EE were observed in skeletal muscles from mice, all these were alleviated by the precondition of TMZ combined with AE. The methods of establishing stress injury models include chemical drugs, ischaemia/reperfusion [58], and EE [59,60]. The first two are usually used to induce specific pathological or organ stress damage, while EE induces comprehensive stress damage in multiple systems and organs, such as severe skeletal muscle injuries [60], mitochondrial and cardiac dysfunction [59], and haematologic system dysregulation [61]. Therefore, EE is more consistent with clinical characteristics compared to the first two methods. EE-related mitochondrial dysfunction resulted in skeletal muscle injury that manifested as increased oxidative stress, cytoskeletal damage, and various markers such as histological changes (Z-line disruption) and biochemical changes (increased CK in serum) [60]. In this study, EE-induced skeletal muscle damage was revealed by increased serum CK activity. And mitochondria oedema, myofibrosis and disordered or even missing sarcomere were observed under EM further confirmed the skeletal muscle injury. Moreover, we detected the decreased activities of SOD, GSH and mit-SOD, while increased activity of MDA in mice underwent fatal-stressor-EE, so verifying the generation of mitochondrial antioxidative enzyme system damage and oxidative stress. These results suggested that EE successfully established the state of fatal stress. Furthermore, all those damages were significantly alleviated in mice treated with TMZ combined with AE, suggesting TMZ combined with AE ameliorated fatal stress-induced skeletal muscle damage and enhanced the anti-fatal stress capacity.

In addition, TMZ combined with AE attenuated skeletal muscle damage in response to EE by improving MQC in skeletal muscle, which was presented as an increased balance between the mitochondrial biogenesis, dynamics, and mitophagy in the fatal stress state. It demonstrated that autophagy is an important protective mechanism against stress injury [62]. Moreover, dysregulation of MQC is the main cause of mitochondrial dysfunction and induction of apoptosis in the stress state [63]. Therefore, maintaining MQC is an important guarantee against stress damage. Recently, Wen Shi et al. [30] have revealed that TMZ preserved the mitochondrial structure, improved respiratory control ratio, and improved mitochondrial biogenesis and mitochondrial dynamics, as demonstrated by an increase expression of PGC-1α, MFN1, and DRP1, in rats with acute myocardial ischaemia. Francesca Molinari et al. [33] also revealed that TMZ upregulated the expression of PGC1-α, mitochondrial protein Tom20, and enhanced succinate dehydrogenase activity in C26-bearing mice. These results confirmed that the attenuation of stress injury by TMZ was closely associated with mitochondrial biogenesis and dynamics. Recently, Song M et al. [55] provided evidence that TMZ alleviated simvastatin-related skeletal muscle injuries by restoring the oxidative phenotype, and reversing the reduction in mitochondrial complex III, membrane potential and CS. In this study, we detected the simultaneous increase in mitochondrial biogenesis, dynamics and mitophagy in mice after EE with intervention of TMZ combined with AE. On one hand, TMZ combined with AE promotes mitochondrial fission to separate EE-induced dysfunctional mitochondria, which undergo mitophagy for efficient energy re-utilization. On the other hand, it enhances mitochondrial biogenesis to increase mitochondria numbers and promotes mitochondrial fusion to maintain MQC. All of these factors contribute to the increase in mitochondrial turnover in skeletal muscle. Moreover, we detected the skeletal muscle ultrastructure under EM, it showed that autophagosomes number significantly increased, while mitochondrial oedema and

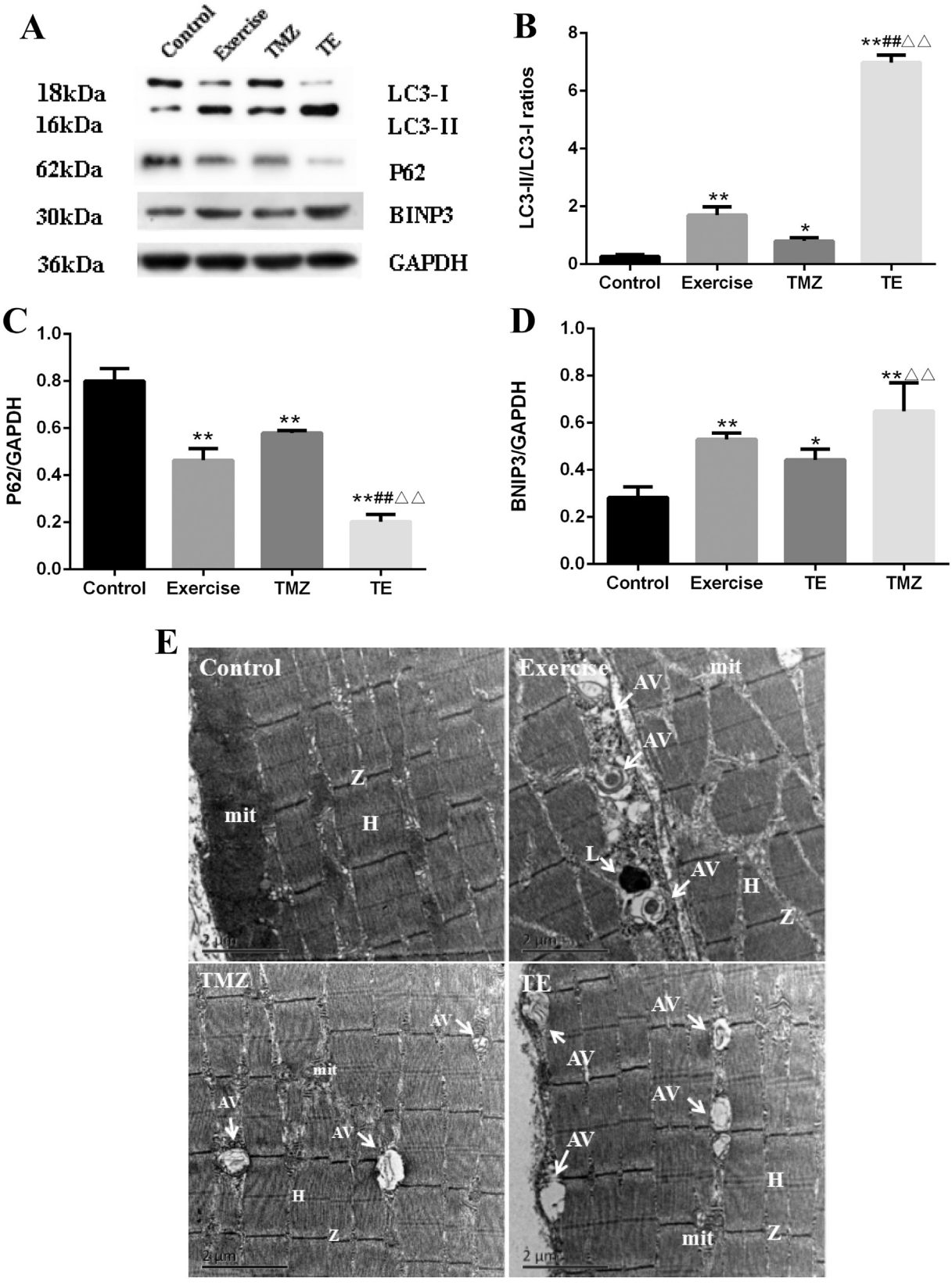

Fig. 5. TMZ and AE, alone or combined, activated mitophagy in the basal state, Representative LC3-II/LC3-I ratios (B), p62 (C), BNIP3 (D) and autophagosomes number under EM from skeletal muscle in mice in the basal state. EM shows that the autophagosomes number (B) in skeletal muscle was significantly increased in the TMZ, exercise and TE groups. mit: mitochondria, AV: autophagic vacuole, Z: Z line, H: H band, L: Lysosome. Bars: A: 5 μm; B: 2 μm or 5 μm; inset, 1 μm. Values are reported as the *mean* ± *SD* (n = 3 muscles per group). $^{\star}P < 0.05$, $^{\star\star}P < 0.01$ *versus Control*; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *versus exercise*; $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$, *versus TMZ*. Bands from the western blotting analysis (A).

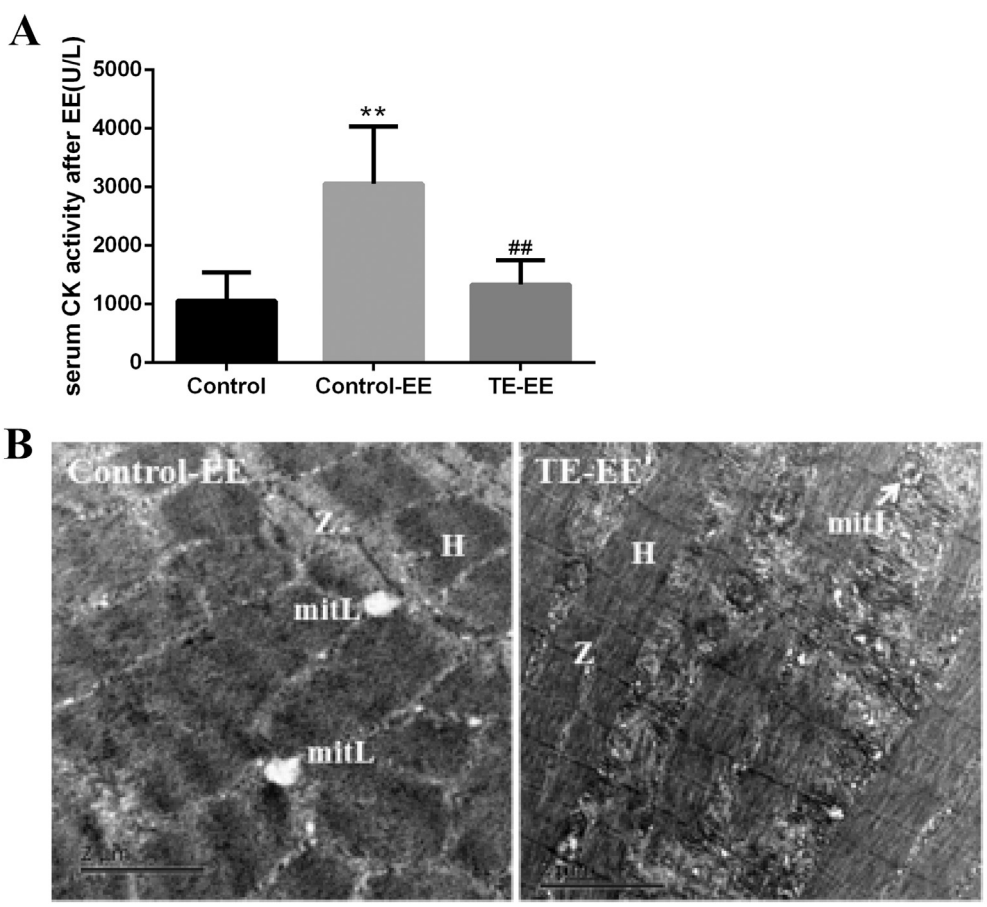

Fig. 6. TMZ combined with AE alleviated skeletal muscle damage caused by fatal stressor-EE. (A) serum CK activity and (B) EM micrographs of skeletal muscles in mice after EE; Values are reported as the *mean* ± *SD* (n = 5). EM image showing the skeletal muscle fibre damage in the control-EE subgroup, presented as myofibre necrosis, disordered sarcoma, blurred or missing Z-lines and H bands in myofibres, and mitochondrial oedema. All of these effects were obviously alleviated in the TE-EE subgroup (B). mitL: Mitochondrial oedema, Z: Z line, H: H band. Bars: A and B: 2 μm. $^{\star}P < 0.05$, $^{\star\star}P < 0.01$ *versus control*; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *versus control-EE*.

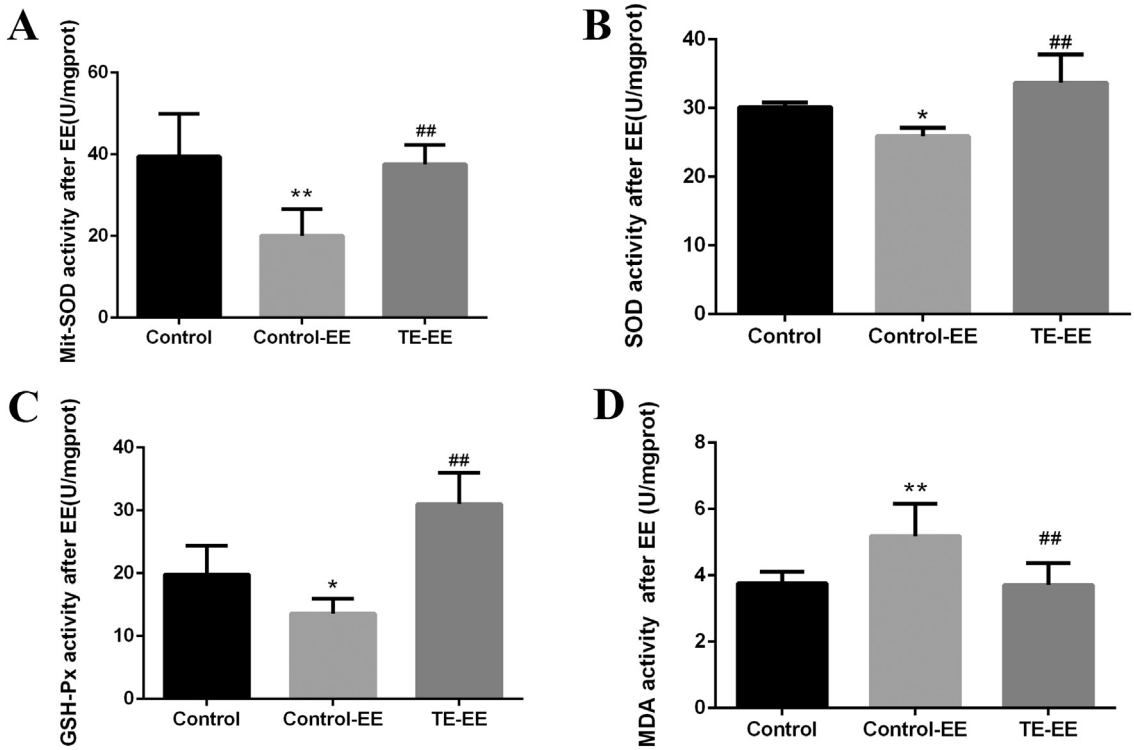

Fig. 7. TMZ combined with AE reversed EE induced mitochondrial oxidative stress and the antioxidative enzyme system damage. The activities of Mit-SOD (A), SOD (B), GSH-Px (C) and MDA (D) in skeletal muscle after EE; Values are reported as the *mean* ± *SD* (n = 5). $^{\star}P < 0.05$, $^{\star\star}P < 0.01$ *versus control*; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *versus control-EE*.

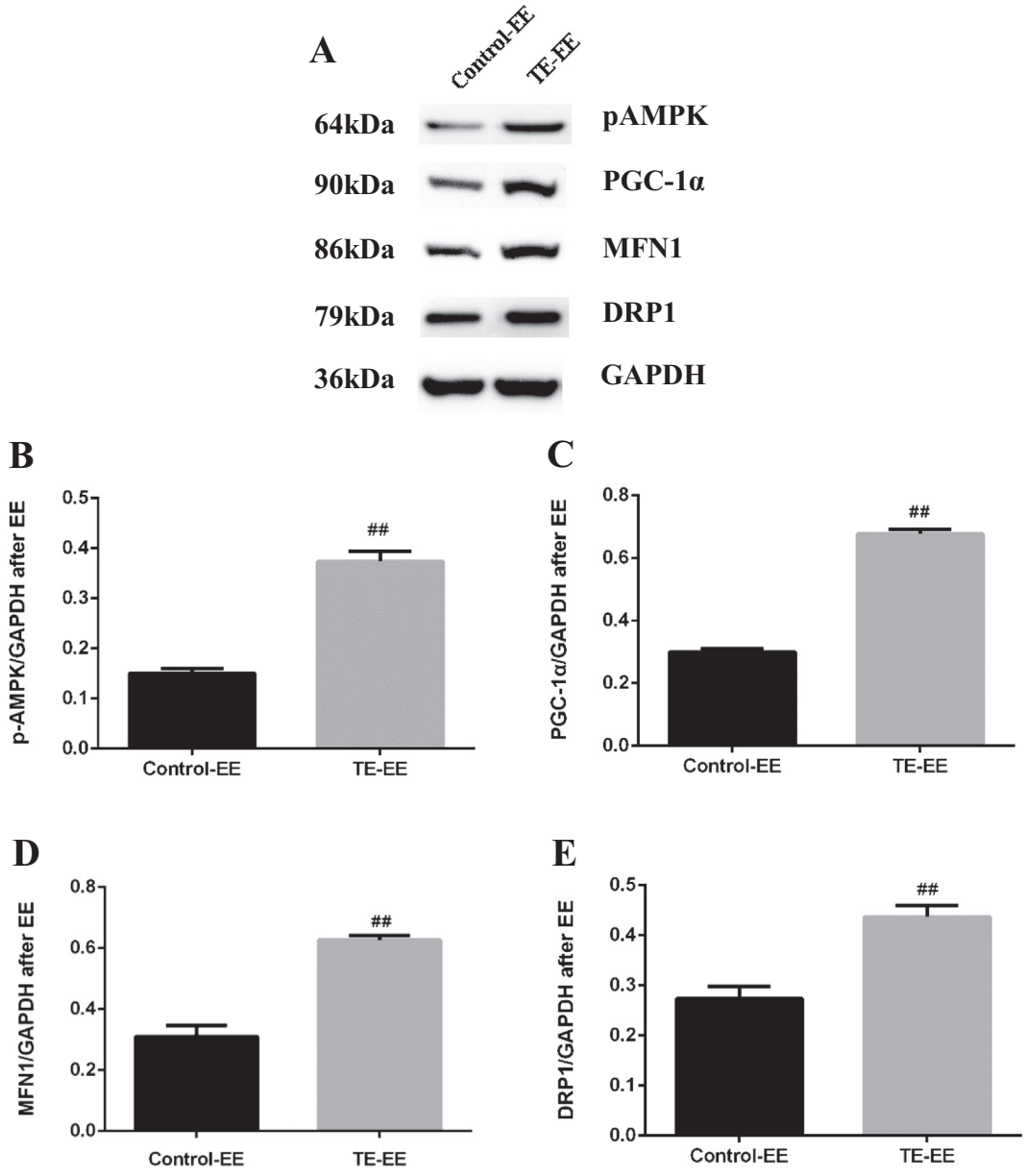

Fig. 8. TMZ combined with AE improved mitochondrial biogenesis and dynamics in skeletal muscle after fatal stressor-EE. The expression levels of pAMPK (B), PGC-1α (C), MFN1 (D) and DRP1 (E) from skeletal muscles in mice after EE; Values are reported as the *mean* ± *SD* (n = 3 muscles per group). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *versus control-EE*. Bands from the western blotting analysis (A).

disordered sarcomere obviously decreased in mice of TE-EE group, as shown in Figs. 6B and 9E. Those observations demonstrated that TMZ combined with AE efficiently maintained MQC and then improved the mitochondrial function in skeletal muscle under fatal stress.

5. Conclusion

The present study for the first time provided evidence that TMZ and TMZ combined with AE improved EC, and the synergistic effects of both interventions were detected. Moreover, the combination of both interventions alleviated fatal stress-induced skeletal muscle damage and enhanced anti-fatal stress ability. Enhancing skeletal muscle MQC may be one of the key mechanisms of AE combined with TMZ that improves EC and anti-fatal stress ability. Further clinical studies are needed to confirm that TMZ combined with AE attenuates disease-related stress and improves the potential to cope with acute coronary syndrome. Though this study did not use an inhibitor or agonist to verify the results, it should be noted that our results still have important implications in reforming cardiac rehabilitation.

Ethical standard statement

Experimental animals were purchased from Hunan SJA Laboratory Animal Co. Ltd. (Changsha, Hunan, China), Licence No. SCXK (Xiang) 2011-0003. The experimental protocol was approved by the Hunan Provincial People's Hospital Animal Care and Use Committee under the guidelines of the Chinese Academy of Sciences (approval ID: SYXK 2015-0013).

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant IDs: 81672262) and Hunan Provincial Science and Technology Department subsidizing project in China (grant

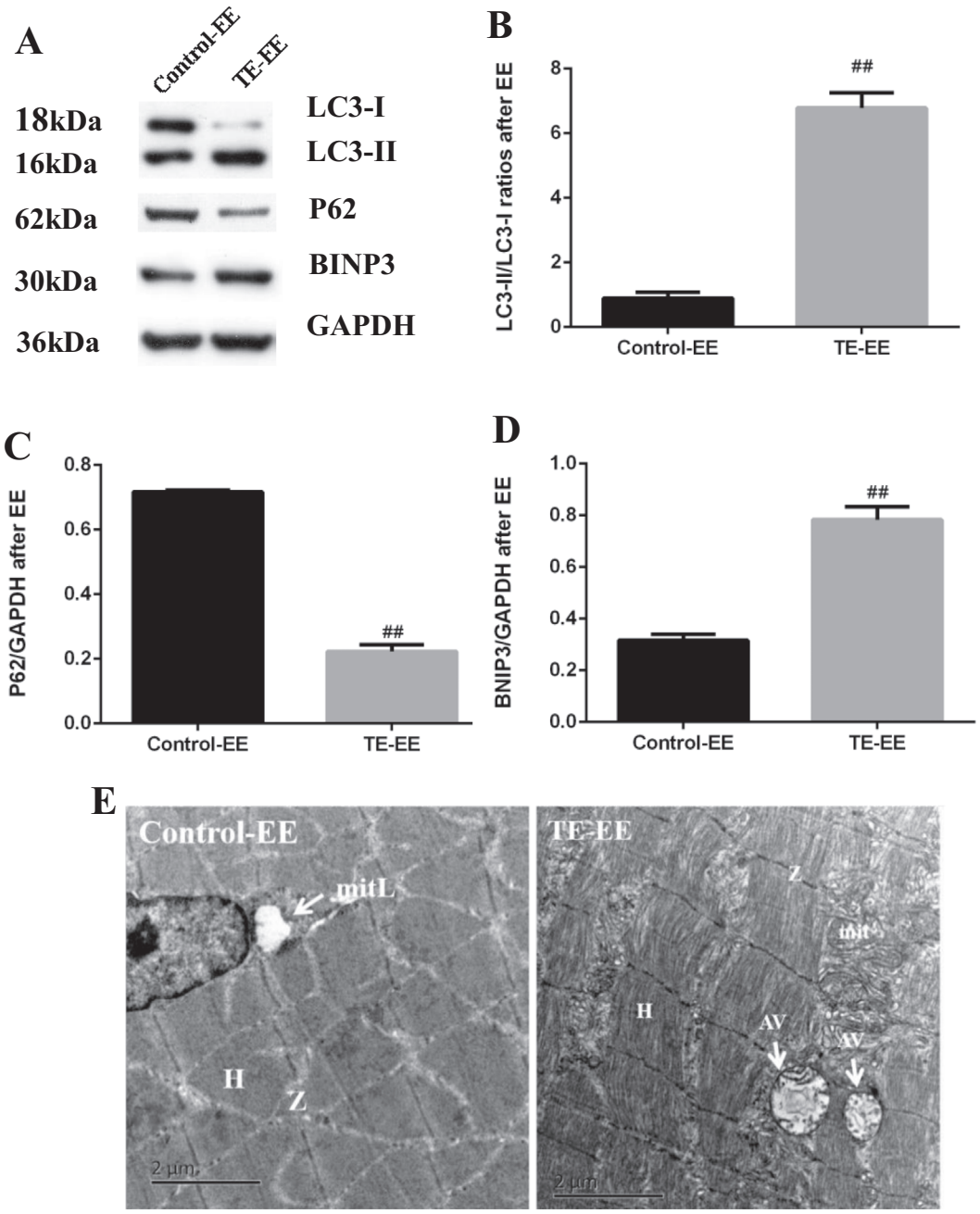

Fig. 9. TMZ combined with AE activated autophagy and mitophagy in skeletal muscle after fatal stressor-EE, the LC3-II/LC3-I ratios (B), and the activities of BINP3 (C) and p62 (D) in skeletal muscle from mice. Values are reported as the *mean* ± *SD* (n = 3 muscles per group). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *versus control-EE*. Bands from the western blotting analysis (A). EM shows that the autophagosomes (E) from skeletal muscle in mice after EE were significantly increased in the TE-EE subgroup. mit: mitochondria, mitL: Mitochondrial oedema, AV: autophagic vacuole, Z: Z line, H: H band. Bars: A and B: 2 μm.

IDs: 13JJ6014).

Conflict of interest

None declared.

References

[1] E.J. Benjamin, et al., Heart disease and stroke statistics-2019 update: a Rreport from the American Heart Association, Circulation 139 (10) (2019) e56–e66.
[2] C. Andersson, R.S. Vasan, Epidemiology of cardiovascular disease in young individuals, Nat. Rev. Cardiol. 15 (2018) 230–240.
[3] R. Ross, et al., Importance of assessing cardiorespiratory fitness in clinical practice: a case for fitness as a clinical vital sign: a scientific statement from the American Heart Association, Circulation 134 (2016) e653–e699.
[4] K.M. Nelson, E.J. Boyko, T. Koepsell, All-cause mortality risk among a national sample of individuals with diabetes, Diabetes Care 33 (2010) 2360–2364.
[5] B.J. Jefferis, P.H. Whincup, O. Papacosta, S.G. Wannamethee, Protective effect of time spent walking on risk of stroke in older men, Stroke 45 (2014) 194–199.
[6] C.D. Lee, S.N. Blair, Cardiorespiratory fitness and stroke mortality in men, Med. Sci. Sports Exerc. 34 (2002) 592–595.
[7] D. Schmid, M.F. Leitzmann, Cardiorespiratory fitness as predictor of cancer mortality: a systematic review and meta-analysis, Annals of Oncology: Official Journal of the European Society for Medical Oncology 26 (2015) 272–278.
[8] J. Li, X. Li, Q. Wang, S. Hu, Y. Wang, F.A. Masoudi, J.A. Spertus, H.M. Krumholz, L. Jiang, P.C.G. China, ST-segment elevation myocardial infarction in China from 2001 to 2011 (the China PEACE-Retrospective Acute Myocardial Infarction Study): a retrospective analysis of hospital data, Lancet 385 (2015) 441–451.
[9] A. Dhopte, R. Bamal, V.K. Tiwari, A prospective analysis of risk factors for pediatric burn mortality at a tertiary burn center in North India, Burns & Trauma 5 (2017) 30.
[10] Mortality GBD, Causes of Death C, Global, regional, and national age-sex specific

all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013, Lancet 385 (2015) 117–171.
[11] Mortality GBD, Causes of Death C, Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015, Lancet 388 (2016) 1459–1544.
[12] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, 2012, CA Cancer J. Clin. 65 (2015) 87–108.
[13] C. Vassalle, S. Bianchi, D. Battaglia, P. Landi, F. Bianchi, C. Carpeggiani, Elevated levels of oxidative stress as a prognostic predictor of major adverse cardiovascular events in patients with coronary artery disease, J. Atheroscler. Thromb. 19 (2012) 712–717.
[14] I. Mordukhovich, E. Wilker, H. Suh, R. Wright, D. Sparrow, P.S. Vokonas, J. Schwartz, Black carbon exposure, oxidative stress genes, and blood pressure in a repeated-measures study, Environ. Health Perspect. 117 (2009) 1767–1772.
[15] K. Harada, S. Suzuki, H. Ishii, T. Aoki, K. Hirayama, Y. Shibata, Y. Negishi, T. Sumi, K. Kawashima, A. Kunimura, Y. Shimbo, Y. Tatami, T. Kawamiya, D. Yamamoto, R. Morimoto, Y. Yasuda, T. Murohara, Impact of skeletal muscle mass on long-term adverse cardiovascular outcomes in patients with chronic kidney disease, Am. J. Cardiol. 119 (2017) 1275–1280.
[16] M.N. Murphy, M. Mizuno, J.H. Mitchell, S.A. Smith, Cardiovascular regulation by skeletal muscle reflexes in health and disease, Am. J. Physiol. Heart Circ. Physiol. 301 (2011) H1191–H1204.
[17] A.M. Joseph, D.A. Hood, Relationships between exercise, mitochondrial biogenesis and type 2 diabetes, Medicine and Sport Science 60 (2014) 48–61.
[18] K.A. Overmyer, C.R. Evans, N.R. Qi, C.E. Minogue, J.J. Carson, C.J. Chermside-Scabbo, L.G. Koch, S.L. Britton, D.J. Pagliarini, J.J. Coon, C.F. Burant, Maximal oxidative capacity during exercise is associated with skeletal muscle fuel selection and dynamic changes in mitochondrial protein acetylation, Cell Metab. 21 (2015) 468–478.
[19] A.R. Anzell, R. Maizy, K. Przyklenk, T.H. Sanderson, Mitochondrial quality control and disease: insights into ischemia-reperfusion injury, Mol. Neurobiol. 55 (2018) 2547–2564.
[20] M.T. Figge, H.D. Osiewacz, A.S. Reichert, Quality control of mitochondria during aging: is there a good and a bad side of mitochondrial dynamics? BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology 35 (2013) 314–322.
[21] N.M. Held, R.H. Houtkooper, Mitochondrial quality control pathways as determinants of metabolic health, BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology 37 (2015) 867–876.
[22] H.M. Ni, J.A. Williams, W.X. Ding, Mitochondrial dynamics and mitochondrial quality control, Redox Biol. 4 (2015) 6–13.
[23] M. Uittenbogaard, A. Chiaramello, Mitochondrial biogenesis: a therapeutic target for neurodevelopmental disorders and neurodegenerative diseases, Curr. Pharm. Des. 20 (2014) 5574–5593.
[24] V.M. Conraads, N. Pattyn, C. De Maeyer, P.J. Beckers, E. Coeckelberghs, V.A. Cornelissen, J. Denollet, G. Frederix, K. Goetschalckx, V.Y. Hoymans, N. Possemiers, D. Schepers, B. Shivalkar, J.U. Voigt, E.M. Van Craenenbroeck, L. Vanhees, Aerobic interval training and continuous training equally improve aerobic exercise capacity in patients with coronary artery disease: the SAINTEX-CAD study, Int. J. Cardiol. 179 (2015) 203–210.
[25] N. Pattyn, E. Coeckelberghs, R. Buys, V.A. Cornelissen, L. Vanhees, Aerobic interval training vs. moderate continuous training in coronary artery disease patients: a systematic review and meta-analysis, Sports Med. 44 (2014) 687–700.
[26] Y. Dun, S. Liu, W. Zhang, M. Xie, L. Qiu, Exercise combined with Rhodiola sacra supplementation improves exercise capacity and ameliorates exhaustive exercise-induced muscle damage through enhancement of mitochondrial quality control, Oxidative Med. Cell. Longev. 2017 (2017) 8024857.
[27] C.A. Dezsi, Trimetazidine in practice: review of the clinical and experimental evidence, Am. J. Ther. 23 (2016) e871–e879.
[28] R. Belardinelli, F. Lacalaprice, E. Faccenda, L. Volpe, Trimetazidine potentiates the effects of exercise training in patients with ischemic cardiomyopathy referred for cardiac rehabilitation, European Journal of Cardiovascular Prevention and Rehabilitation: Official Journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology 15 (2008) 533–540.
[29] C.P. McCarthy, K.V. Mullins, D.M. Kerins, The role of trimetazidine in cardiovascular disease: beyond an anti-anginal agent, European Heart Journal. Cardiovascular Pharmacotherapy 2 (2016) 266–272.
[30] W. Shi, W. Shangguan, Y. Zhang, C. Li, G. Li, Effects of trimetazidine on mitochondrial respiratory function, biosynthesis, and fission/fusion in rats with acute myocardial ischemia, Anatolian Journal of Cardiology 18 (2017) 175–181.
[31] J. Kuzmicic, V. Parra, H.E. Verdejo, C. Lopez-Crisosto, M. Chiong, L. Garcia, M.D. Jensen, D.A. Bernlohr, P.F. Castro, S. Lavandero, Trimetazidine prevents palmitate-induced mitochondrial fission and dysfunction in cultured cardiomyocytes, Biochem. Pharmacol. 91 (2014) 323–336.
[32] F. Molinari, F. Pin, S. Gorini, S. Chiandotto, L. Pontecorvo, F. Penna, E. Rizzuto, S. Pisu, A. Musaro, P. Costelli, G. Rosano, E. Ferraro, The mitochondrial metabolic reprogramming agent trimetazidine as an 'exercise mimetic' in cachectic C26-bearing mice, J. Cachexia. Sarcopenia Muscle 8 (2017) 954–973.
[33] L. Gatta, L. Vitiello, S. Gorini, S. Chiandotto, P. Costelli, A.M. Giammarioli, W. Malorni, G. Rosano, E. Ferraro, Modulating the metabolism by trimetazidine enhances myoblast differentiation and promotes myogenesis in cachectic tumor-bearing c26 mice, Oncotarget 8 (2017) 113938–113956.
[34] S. Freimann, M. Scheinowitz, D. Yekutieli, M.S. Feinberg, M. Eldar, G. Kessler-Icekson, Prior exercise training improves the outcome of acute myocardial infarction in the rat. Heart structure, function, and gene expression, J. Am. Coll. Cardiol. 45 (2005) 931–938.
[35] R.E. Wu, W.C. Huang, C.C. Liao, Y.K. Chang, N.W. Kan, C.C. Huang, Resveratrol protects against physical fatigue and improves exercise performance in mice, Molecules 18 (2013) 4689–4702.
[36] S.J. Moat, T. Korpimaki, P. Furu, H. Hakala, H. Polari, L. Merio, P. Makinen, I. Weeks, Characterization of a blood spot creatine kinase skeletal muscle isoform immunoassay for high-throughput newborn screening of Duchenne muscular dystrophy, Clin. Chem. 63 (2017) 908–914.
[37] T.L. Cheng, C.C. Liao, W.H. Tsai, C.C. Lin, C.W. Yeh, C.F. Teng, W.T. Chang, Identification and characterization of the mitochondrial targeting sequence and mechanism in human citrate synthase, J. Cell. Biochem. 107 (2009) 1002–1015.
[38] L. Liu, K. Sakakibara, Q. Chen, K. Okamoto, Receptor-mediated mitophagy in yeast and mammalian systems, Cell Res. 24 (2014) 787–795.
[39] D.J. Klionsky, et al., Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition), Autophagy 12 (2016) 1–222.
[40] T. Zhang, L. Xue, L. Li, C. Tang, Z. Wan, R. Wang, J. Tan, Y. Tan, H. Han, R. Tian, T.R. Billiar, W.A. Tao, Z. Zhang, BNIP3 protein suppresses PINK1 kinase proteolytic cleavage to promote mitophagy, J. Biol. Chem. 291 (2016) 21616–21629.
[41] M.L. Seibenhener, Y. Du, M.T. Diaz-Meco, J. Moscat, M.C. Wooten, M.W. Wooten, A role for sequestosome 1/p62 in mitochondrial dynamics, import and genome integrity, Biochim. Biophys. Acta 1833 (2013) 452–459.
[42] E. Ferraro, F. Pin, S. Gorini, L. Pontecorvo, A. Ferri, V. Mollace, P. Costelli, G. Rosano, Improvement of skeletal muscle performance in ageing by the metabolic modulator Trimetazidine, J. Cachexia. Sarcopenia Muscle 7 (2016) 449–457.
[43] V. Romanello, M. Sandri, Mitochondrial quality control and muscle mass maintenance, Front. Physiol. 6 (2015) 422.
[44] H.B. Suliman, C.A. Piantadosi, Mitochondrial quality control as a therapeutic target, Pharmacol. Rev. 68 (2016) 20–48.
[45] E.A. de Lima, L.G.O. de Sousa, S.T.A.A. de, A.G. Marshall, N.E. Zanchi, J.C.R. Neto, Aerobic exercise, but not metformin, prevents reduction of muscular performance by AMPk activation in mice on doxorubicin chemotherapy, J. Cell. Physiol. 233 (12) (2018) 9652–9662.
[46] L. Lantier, J. Fentz, R. Mounier, J. Leclerc, J.T. Treebak, C. Pehmoller, N. Sanz, I. Sakakibara, E. Saint-Amand, S. Rimbaud, P. Maire, A. Marette, R. Ventura-Clapier, A. Ferry, J.F. Wojtaszewski, M. Foretz, B. Viollet, AMPK controls exercise endurance, mitochondrial oxidative capacity, and skeletal muscle integrity, FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology 28 (2014) 3211–3224.
[47] H.M. O'Neill, S.J. Maarbjerg, J.D. Crane, J. Jeppesen, S.B. Jorgensen, J.D. Schertzer, O. Shyroka, B. Kiens, B.J. van Denderen, M.A. Tarnopolsky, B.E. Kemp, E.A. Richter, G.R. Steinberg, AMP-activated protein kinase (AMPK) beta1beta2 muscle null mice reveal an essential role for AMPK in maintaining mitochondrial content and glucose uptake during exercise, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 16092–16097.
[48] P. Chrusciel, J. Rysz, M. Banach, Defining the role of trimetazidine in the treatment of cardiovascular disorders: some insights on its role in heart failure and peripheral artery disease, Drugs 74 (2014) 971–980.
[49] M. Hey-Mogensen, K. Hojlund, B.F. Vind, L. Wang, F. Dela, H. Beck-Nielsen, M. Fernstrom, K. Sahlin, Effect of physical training on mitochondrial respiration and reactive oxygen species release in skeletal muscle in patients with obesity and type 2 diabetes, Diabetologia 53 (2010) 1976–1985.
[50] A. Shirakabe, P. Zhai, Y. Ikeda, T. Saito, Y. Maejima, C.P. Hsu, M. Nomura, K. Egashira, B. Levine, J. Sadoshima, Drp1-dependent mitochondrial autophagy plays a protective role against pressure overload-induced mitochondrial dysfunction and heart failure, Circulation 133 (2016) 1249–1263.
[51] M. Song, K. Mihara, Y. Chen, L. Scorrano, G.W. Dorn 2nd, Mitochondrial fission and fusion factors reciprocally orchestrate mitophagic culling in mouse hearts and cultured fibroblasts, Cell Metab. 21 (2015) 273–286.
[52] G. Twig, A. Elorza, A.J. Molina, H. Mohamed, J.D. Wikstrom, G. Walzer, L. Stiles, S.E. Haigh, S. Katz, G. Las, J. Alroy, M. Wu, B.F. Py, J. Yuan, J.T. Deeney, B.E. Corkey, O.S. Shirihai, Fission and selective fusion govern mitochondrial segregation and elimination by autophagy, EMBO J. 27 (2008) 433–446.
[53] H.R. Waterham, J. Koster, C.W. van Roermund, P.A. Mooyer, R.J. Wanders, J.V. Leonard, A lethal defect of mitochondrial and peroxisomal fission, N. Engl. J. Med. 356 (2007) 1736–1741.
[54] C. Vitale, G. Marazzi, F. Pelliccia, M. Volterrani, E. Cerquetani, I. Spoletini, G. Mercuro, S. Bonassi, V. Dall'Armi, M. Fini, G.M. Rosano, Trimetazidine improves exercise performance in patients with peripheral arterial disease, Pharmacol. Res. 63 (2011) 278–283.
[55] M. Song, F.F. Chen, Y.H. Li, L. Zhang, F. Wang, R.R. Qin, Z.H. Wang, M. Zhong, M.X. Tang, W. Zhang, L. Han, Trimetazidine restores the positive adaptation to exercise training by mitigating statin-induced skeletal muscle injury, J. Cachexia. Sarcopenia Muscle 9 (2018) 106–118.
[56] S. Jager, C. Handschin, J. St-Pierre, B.M. Spiegelman, AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 12017–12022.
[57] C. Canto, L.Q. Jiang, A.S. Deshmukh, C. Mataki, A. Coste, M. Lagouge, J.R. Zierath, J. Auwerx, Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle, Cell Metab. 11 (2010) 213–219.
[58] J. Liu, Q. Chen, Z. Jian, X. Xiong, L. Shao, T. Jin, X. Zhu, L. Wang, Daphnetin protects against cerebral ischemia/reperfusion injury in Mmice via inhibition of TLR4/NF-kappaB signaling pathway, Biomed. Res. Int. 2016 (2016) 6. Article ID 2816056.
[59] K. Ljones, H.O. Ness, K. Solvang-Garten, S.E. Gaustad, M. Andre Hoydal, Acute exhaustive aerobic exercise training impair cardiomyocyte function and calcium handling in Sprague-Dawley rats, PLoS One 12 (2017) e0173449.

[60] M. Behringer, J. Montag, A. Franz, M.L. McCourt, J. Mester, K.K. Nosaka, Exhaustive exercise—a near death experience for skeletal muscle cells? Med. Hypotheses 83 (2014) 758–765.
[61] H. Zhang, H. Zhang, S. Lu, H. Bai, Y. Gu, Effects of preventative moxibustion on AMPK and mTOR in myocardial tissue in rats with exhaustive exercise, Zhongguo Zhen Jiu = Chinese Acupuncture & Moxibustion 37 (2017) 521–526.
[62] Z. Lei, M. Deng, Z. Yi, Q. Sun, R.A. Shapiro, H. Xu, T. Li, P.A. Loughran, J.E. Griepentrog, H. Huang, M.J. Scott, F. Huang, T.R. Billiar, cGAS-mediated autophagy protects the liver from ischemia-reperfusion injury independently of STING, Am. J. Physiol. Gastrointest. Liver Physiol. 314 (2018) G655–G667.
[63] Y. Lee, I. Kwon, Y. Jang, W. Song, L.M. Cosio-Lima, M.H. Roltsch, Potential signaling pathways of acute endurance exercise-induced cardiac autophagy and mitophagy and its possible role in cardioprotection, The Journal of Physiological Sciences: JPS 67 (2017) 639–654.