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ORIGINAL ARTICLE

Effect of high-intensity interval and endurance training with MitoQ on mitochondrial dynamics in rat muscle

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ABSTRACT

Background and Aim: Mitochondria play an important role in signaling and metabolic pathways in skeletal muscle. In this study, the effects of MitoQ supplementation alone and in combination with endurance training (ET) or high-intensity interval training (HIIT) were investigated in relation to the process of mitochondrial quality control in the gastrocnemius muscle of male rats.

Methods: Animals were assigned into 6 groups (n = 7): Control, MitoQ, ET, ET + MitoQ, HIIT, and HIIT + MitoQ. The gene and protein expression were quantified using real-time polymerase chain reaction ($2^{-\Delta\Delta CT}$) and Western blot analysis, respectively. Statistical analysis was performed using one-way analysis of variance.

Results: ET significantly increased protein expression of dynamin-related protein 1 (DRP1) and mitofusin1 (MFN1) and gene expression of optic atrophy Type 1 (*Opa1*) in skeletal muscle, when compared to the control group ($p < 0.001$). HIIT only increased MFN1 protein expression compared to the control group ($p < 0.0001$). MitoQ in combination with HIIT significantly increased protein expression of DRP1 and MFN1 compared to MitoQ alone ($p < 0.01$).

Conclusion: In sum, exercise training can affect mitochondrial dynamics by changing the factors involved in the fission and fusion process, and ET can improve training capacity in skeletal muscle by modulating expression of OPA1 and MFN1. While MitoQ supplementation alone did not significantly alter the mitochondrial fission-fusion process, its combination with HIIT appeared to elevate the expression of DRP1, suggesting a potential synergistic effect that warrants further investigation. Future studies should delve into the mechanisms by which MitoQ and exercise-induced stress affect mitochondrial quality control, particularly in the context of redox modulation and signaling pathways that govern mitochondrial plasticity.

Relevance for Patients: Combining MitoQ with exercise training may enhance mitochondrial function, potentially improving muscle health in patients.

1. Introduction

Regarded as dynamic organelles, mitochondria change their shape and structure in response to various metabolic stimuli. Mitochondrial structure is mainly regulated by fusion and fission cycles. These processes are tightly regulated to ensure a balance. Dysregulated fusion and fission of mitochondria is an important mechanism in the development of some diseases [1]. Mitochondrial dysfunction results in chronic

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inflammation, which leads to a vicious cycle between chronic inflammation and mitochondrial reactive oxygen species (ROS) production [2].

Exercise training provides a powerful boost to initiate the signaling pathways described above, which eventually create strong phenotypic changes in the mitochondria and improve the quantity and quality of the organelle network, leading to greater muscle health [3]. Exercise triggers an adaptive response through redox signaling that increases mitochondrial function (a combination of quality and quantity), boosting metabolism and antioxidant stores, not only allowing the organism to better control inflammation but also making it more resistant to most stressors [4]. In addition, mitochondria can be involved in the progression of sarcopenia as they are critical controllers of an assortment of variables that contribute to the etiology of the condition, such as ATP provision, oxidative stress, and apoptosis, as well as inflammation and calcium ions (Ca^{2+}) handling [5]. Regular aerobic exercise can relieve inveterate irritation in skeletal muscle by activating mitochondrial quality control processes to improve organelle phenotypes, with the possibility of reducing the release of mitochondrial damage-associated molecular patterns (mtDAMPs). This could attenuate inflammasome activation and counteract the detrimental effects of chronic inflammation on muscle development and function [6].

Mitochondrial stress, a consequence of stimuli such as exercise training, leads to the release of significant stress signals including ROS and Ca^{2+}, which are crucial in regulating cellular signaling cascades [7]. However, excessive mitochondrial stress or failure of the adaptive response may result in irreversible mitochondrial damage and the release of apoptotic signals, ultimately triggering cellular apoptosis [7]. During the apoptosis process, mitochondria are influenced by dynamic changes in various proteins, such as dynamin-related protein 1 (Drp1) and the apoptotic regulators BAX and BCL-2 [8]. In addition, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is essential for regulating key factors that orchestrate mitochondrial dynamics and mitophagy, such as mitofusin 2 (MFN2), DRP1, PTEN-induced putative kinase protein 1 (PINK1), and parkin [9]. Furthermore, an interplay between PGC-1α and nuclear factor erythroid 2-related factor 2 (NRF2) is crucial for modulating mitochondrial stress adaptations, highlighting their roles in promoting cell survival and reducing oxidative stress [10]. Inhibiting mitochondrial fission can delay downstream caspase activation and subsequent apoptosis, while overexpression of mitofusin 1 (MFN1) or MFN2 may postpone apoptotic cell death. The fusion process permits the integration of fragmented, viable mitochondria back into the mitochondrial network [11]. Mitochondrial fusion is regulated by two members of the large GTPase family, MFN1 and MFN2 [12]. The genetic deletion of mitofusins leads to the accumulation of dysfunctional mitochondria and subsequent cell damage [13]. MFN2 expression can be downregulated by growth factors, cytokines, and lipid availability, but upregulated by exercise and energy consumption [14]. In addition, several intracellular pathways, including those associated with cell cycle progression, mitochondrial bioenergetics maintenance, apoptosis, and autophagy, affect MFN2 expression [15]. Moreover, mitochondrial fission is influenced by proteins such as mitochondrial fission 1 protein (FIS1) and DRP1, while the fusion process is mediated by the optic atrophy 1 (OPA1) and mitofusins protein family [16].

MitoQ, a mitochondria-targeted antioxidant supplement, scavenges free radicals, reduces oxidant production and lipid peroxidation, and removes peroxide nitrite [17], being ~800-fold more effective than untargeted antioxidants. It condenses on the matrix surface of the inner mitochondrial membrane and exerts antioxidative effects by oxidizing ubiquinol to ubiquinone [18]. It has been shown that chronic administration of MitoQ ameliorates mitochondrial ROS production in the skeletal muscles of middle-aged men [19]. The MitoQ supplement is an advanced orally used antioxidant that protects the mitochondria as the energy production site. MitoQ is designed to accumulate in the mitochondrial matrix and ultimately exert beneficial effects by electron reduction and free radical attenuation [20]. MitoQ reduces the production of ROS and imbalance in mitochondrial membrane potential [21], prevents endothelial cell death, and normalizes vascular function in various diseases [22]. However, the effectiveness of MitoQ supplementation is not fully understood. In another study, the effect of MitoQ in combination with endurance training (ET) on male athletes showed that MitoQ supplementation increases the antioxidant capacity of skeletal muscle [23].

Scientists have utilized a variety of exercise training protocols to challenge skeletal muscle to determine the effect on acute mitochondrial signaling and chronic mitochondrial adaptation. Moore *et al.* [24] showed that regulators of mitochondrial fission, *Fis1*, and *Dnm1L* (encodes the protein DRP1) were upregulated in the skeletal muscle of mice after ET for 90 min, although only *Fis1* expression remained elevated during the 3-h recovery period, and *Dnm1L* expression returned to baseline (90-min exercise training + 3-h rest). In contrast to *Fis1*, mitochondrial fission factor (*Mff*) expression was reduced during acute exercise and this reduction in expression reached statistical significance during the 3-h recovery period (90-min exercise training + 3-h rest) [24]. Endurance exercise (one session) can be sufficient to induce changes in the mitochondrial life cycle including mitochondrial fission signaling through Drp1. Furthermore, lacking Drp1 reduced exercise performance and altered training-induced adaptations [24]. In mouse models of fission-fusion incompetence, researchers have shown that impaired dynamic flux of mitochondrial remodeling is associated with derangements in metabolism and insulin sensitivity. Because the processes of mitochondrial fission, fusion, biogenesis, and quality control are interdependent, strategies aimed at enhancing mitochondrial lifecycle flux capacity may be effective in combating diseases associated with metabolic dysfunction.

Mitochondrial health and function are critical for overall cellular performance, and exercise training is a well-known stimulus that can enhance mitochondrial quality. The effects of different training modalities, specifically ET and HIIT, on mitochondrial dynamics remain largely unexplored. In this

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study, we hypothesize that MitoQ supplementation, both as an isolated treatment and combined treatment with ET and HIIT, will significantly influence mitochondrial dynamics within the gastrocnemius muscle of male rats. Moreover, we propose that the distinct characteristics of each training modality may differentially impact mitochondrial dynamic when paired with MitoQ supplementation. This study aims to provide deeper insights into how these interventions can enhance mitochondrial function.

2. Materials and Methods

2.1. Animals

Forty-two male Wistar rats, each weighing 200 ± 10 g and aged 8 weeks, were obtained from the Physiology Research Center (Karman, Iran) and maintained under standard conditions as follows: 12-h light/dark cycle; 23 ± 2°C; and humidity of 55 ± 10%. Animals were randomly divided into 6 groups ($n = 7$): (i) Untreated control (CTL); (ii) MitoQ, which received 250 μM MitoQ in drinking water for 8 weeks; (iii) ET, which performed ET; (iv) ET + MitoQ; (v) HIIT, which performed high-intensity interval training; and (vi) HIIT + MitoQ. In the ET groups, the rats were trained on a treadmill for 8 weeks, 5 days/week, 50 min/day. Animals in the HIIT groups performed training (80 – 90% of V_{max} in 2-min intervals) 5 days/week for 8 weeks by running on a treadmill. The animal procedure was approved by the Animal Care Committee of the Ethics Committee of Kerman University of Medical Sciences (IR.KMU.REC.1400.292). All methods were performed in adherence to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines 2.0.

2.2. MitoQ treatment

MitoQ (MitoQ Ltd, New Zealand) was given to animals at a dose of 250 μM in drinking water [25]. To assess the targeting of MitoQ in tissues, the concentration of MitoQ was measured in the whole lysate tissues by means of high-performance liquid chromatography-mass spectrometry (HPLC-MS) [26]. For HPLC-MS calibration, we used the MitoQ internal standard (MRC Mitochondrial Biology Unit and Department of Medicine, University of Cambridge), and the tissue levels of MitoQ in the CTL group and MitoQ group were 0 and 5.68 ± 0.81 pmol/100 mg protein, respectively.

2.3. Training protocol

The rats' familiarization to adapt to the training on treadmill lasted 2 weeks, with each session set at a speed of 15 m/min for 15 min. The intensity of exercise training was calculated by measuring V_{max}. For calculation of the intensity, the speed test started with a warm-up of 10 m/min and then increased (3 m/min) till exhaustion [27]. Blood lactate levels were measured by a lactometer (Lactate Scout Company/Code: 37, Germany) directly after incremental test, and levels above 6 mmol/L were considered high intensity [28]. For HIIT, each session consisted of ten 2-min work bouts/day at 80 – 90% of V_{max} and separated by 2-min rest periods, 5 days/week for 8 weeks [29]. In the ET groups, rats were trained on a treadmill for 8 weeks, 5 days/week, 50 min/day (speed at the last week: 65 – 70% of V_{max}). The iso-distance method was used for two protocols to unify two types of the training protocol. Forty-eight hours after the end of the final training session, animals were anesthetized and sacrificed. The gastrocnemius muscle was extracted and washed with cold normal saline, frozen by liquid nitrogen, and stored at −80°C for real-time polymerase chain reaction (PCR) and Western blotting.

2.4. Western blotting

The protein levels of DRP1 and MFN1 were measured by means of Western blotting. Initially, 20 mg of skeletal muscle tissue was homogenized in cold RIPA (radioimmunoprecipitation assay) buffer containing 0.5% sodium deoxycholate, 150 mM sodium chloride (NaCl), 1.0 mM EDTA, 0.1%, 50 mM Tris-HCl, sodium dodecyl sulfate (SDS), and protease inhibitor with a pH of 7.4. After homogenization, the mixture was centrifuged (15000 rpm, 20 min, 4°C) and the supernatant was collected. Protein levels in the supernatant were determined using the Bradford method. Equal volumes of sample buffer (composed of 62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 0.005% bromophenol blue, and 5% β-mercaptoethanol) were mixed with the protein sample from the supernatant. The mixture was incubated at 95°C for 5 min to denature the proteins, after which each sample was loaded into the appropriate well of a 10% SDS-PAGE gel (running buffer: 25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3). Following electrophoresis, proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane using a transfer buffer (20% methanol, 25 mM Tris, 192 mM glycine, pH 8.3) under the following conditions: 200 mA for 60 min at 4°C. The blocking step was performed with a blocking solution consisting of nonfat dried milk (5% w/v) diluted in Tris-buffered saline with Tween 20 (TBST; 20 mM Tris, 150 mM NaCl, 0.1% Tween 20, pH 7.6). After blocking, the membrane was washed five times for 5 min each with TBST. For antibody detection, the PVDF membrane was incubated with primary antibodies: anti-DRP1 (sc-271583, Santa Cruz Biotechnology) and anti-MFN1 (sc-166644, Santa Cruz Biotechnology). Following five washes (5 min each) with TBST, the blots were incubated with a peroxidase-conjugated secondary antibody. After additional washing, the blots were visualized using a chemiluminescent detection system. The amount of protein was measured by quantitative density analysis and compared to β-actin (detected with anti-β-actin; sc-47778, Santa Cruz Biotechnology) as a control by Image J software [30].

2.5. Total RNA extraction and quantitative realtime PCR

The real-time PCR method was used to determine the relative expression of the genes. Total RNA was extracted from the skeletal muscle tissues using EZ-10 Spin Column Total RNA Mini-preps Kit (Bio Basic, Canada, Cat number, BS1361-SK8655). The process of RNA extraction typically involves the destruction of tissue in a chemical environment that simultaneously inactivates ribonucleases and the subsequent capture of RNA molecules using columns while leaving other

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molecules to pass through the filter. In the final step, the RNAs in the column were eluted from the column using an elution buffer and collected in sterile tubes. Then, the concentration and purity of the total extracted RNA were determined using a nanodrop instrument (Nanodrop-KLAB, South Korea). The cDNA was synthesized from total RNAs while to inhibit the RNase enzyme, RNasin (RNase inhibitor) was added to the reaction mixture (Parstous Biotechnology, Iran). Real-time PCR was performed on cDNA with polymerase enzyme, Master Mix Green (Ampliqon, Denmark), and specific primers (Table 1) for the target genes. The 18s rRNA gene was used as the housekeeping gene. After real-time PCR, Ct values were obtained for both target and reference genes. The ΔCt value indicates the difference of Ct values between the target gene and the housekeeping gene. The $2^{-\Delta\Delta CT}$ formula was used to determine relative gene expression [31].

2.6. Statistical analysis

Data are presented as mean ± standard deviation (SD). After assessing data normality with the Kolmogorov–Smirnov test, one-way analysis of variance (ANOVA) was used for comparisons between groups, followed by Tukey's post-hoc test. $p < 0.05$ was considered significant. In addition, seven rats were allocated to each group to achieve the effect size (f) of 9.044 and a study power of 95% (1-β error probe).

3. Results

Compared to the control group, the ET groups exhibited significantly increased protein levels of DRP1 ($F = 39.09$, $p < 0.0001$) and MFN1 ($F = 18.64$, $p = 0.0003$), as well as *Opa1* gene expression ($F = 4.96$, $p = 0.02$) in skeletal muscle (Figures 1A and B, and Figure 2). Meanwhile, HIIT only increased MFN1 protein levels ($F = 18.64$, $p < 0.0001$) (Figure 1B). MitoQ did not have a significant effect on the gene and protein expression (Figures 1-4). However, its combination with both exercise modalities caused significant effects. In the MitoQ-treated groups separately receiving ET or HIIT, DRP1 protein levels increased, when compared to the levels in the MitoQ group ($F = 39.09$, $p = 0.0006$; $F = 39.09$, $p < 0.0001$; respectively) (Figure 1A). Furthermore, we found that only the HIIT + MitoQ group exhibited increased MFN1 protein levels compared to the MitoQ group ($F = 18.64$, $p < 0.0001$) (Figure 1B). The gene expression of *Opa1*, *Fis1*, and *Mfn2* was unaffected by either the HIIT + MitoQ or ET + MitoQ regimens (Figures 2-4).

Table 1. Primers' sequence used in this study

Genes	Sequences (5' – 3')
Fis1	Forward: CAAGGAACTGGAGCGGCTCATT
	Reverse: GACACAGCAAGTCCGATGAGT
Opa1	Forward: CAGCTGGCAGAAGATCTCAAG
	Reverse: CATGAGCAGGATTTTGACACC
Drp1	Forward: CCAGGAATGACCAAGGTCCC
	Reverse: CCTCGTCCATCAGGTCCAAC
Mfn1	Forward: TGGGGAGGTGCTGTCTCGGA
	Reverse: ACCAATCCCGCTGGGGAGGA
Mfn2	Forward: AGCCTGGTGAGTGTGATGTG
	Reverse: CTCCGTGGTGACATCGATCC
B-actin	Forward: CCAGAGGCGTACAGGGATAG
	Reverse: CCAACCGCGAGAAGATGA

4. Discussion

The results of this study demonstrated that MitoQ alone did not change the expression of factors involved in mitochondrial dynamics, but combination of MitoQ with exercise training led to the modulation of their expression. Compared to HIIT, ET caused more remarkable changes in gene and protein expression in skeletal muscle. ET significantly increased protein expression of DRP1 and MFN1 as well as *Opa1* gene expression, while HIIT only elevated the MFN1 protein level. These findings suggest that in skeletal muscle, ET affects proteins involved in both mitochondrial fusion and fission but HIIT affects only factors involved in mitochondrial fusion.

The increase in MFN2 levels improves muscle performance following appropriate intervention. Intermittent aerobic exercise training improves energy access and reduces oxidative stress damage by increasing the expression of MFN2 and OPA1 [32]. It seems that when the rats performed ET, due to aerobic conditions (in this case, most energy production is produced by mitochondria through aerobic oxidation), the proteins related to both fusion and fission processes increased in levels and helped to improve the dynamics of mitochondria to ensure optimal mitochondrial capacity, whereas through HIIT, due to the presence of alternating aerobic and anaerobic conditions, metabolic and energy requirements were adjusted accordingly. Mitochondrial dynamics is dependent on metabolic conditions [33]. Due to this, mitochondria undergo biogenesis and fusion through PGC-1α [34,35] to meet energy requirements, and at the same time, the progression towards fission happens to remove damaged mitochondria [36]. While our results indicated that MitoQ alone did not significantly alter the expression of factors involved in mitochondrial dynamics, it is important to highlight that the interplay between exercise training and MitoQ treatment reveals critical insights into this relationship. Specifically, our findings demonstrated that the combination of exercise training and MitoQ led to enhanced expression of certain proteins involved in mitochondrial dynamics, particularly in the context of ET, which significantly increased DRP1 and MFN1 protein levels and *Opa1* gene expression [31]. This suggests that while MitoQ may not independently modify mitochondrial dynamics, it has the potential to influence these processes when combined with an exercise regimen. Furthermore, our results indicated that ET promotes both mitochondrial fusion and fission, aligning with metabolic needs, while the effects of HIIT were more central to mitochondrial fusion, reflecting a subtle understanding of how varying exercise protocols impact mitochondrial dynamics [32].

It has been reported that in skeletal muscle tissues, a session of aerobic training, despite improving mitochondrial function, does not affect mitochondrial dynamics [37]. This is in contrast with the findings of our study, and it may be due to the longer duration of the training period in our study compared to acute

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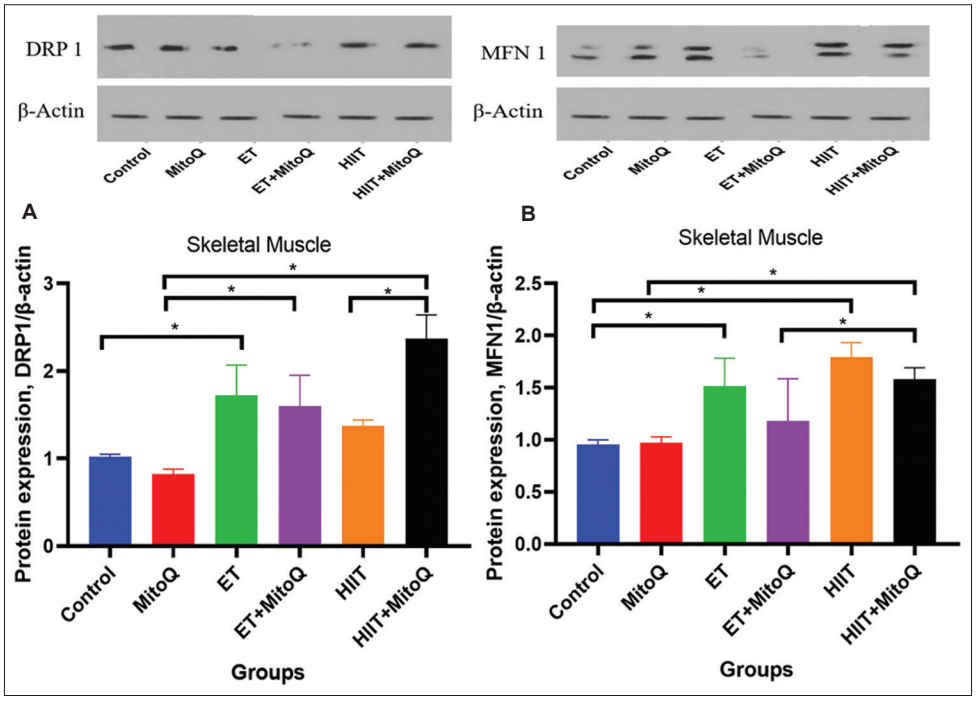

Figure 1. Protein expression of DRP1 in skeletal muscles. (A) DRP1 protein expression in different groups. (B) MFN1 protein expression in different groups. *$p < 0.05$
Abbreviations: ET: Endurance training; HIIT: High-intensity interval training

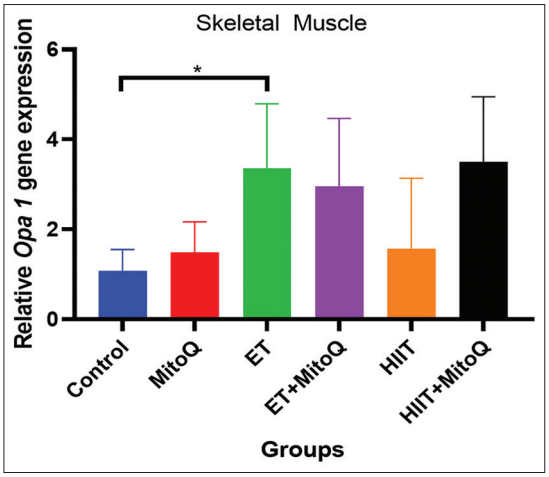

Figure 2. Quantitation of *Opa1* gene expression in skeletal muscles (mean ± SD). *$p < 0.05$
Abbreviations: ET: Endurance training; HIIT: High-intensity interval training

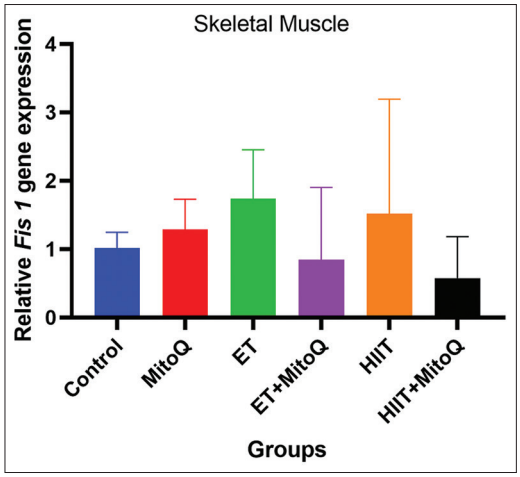

Figure 3. Quantitation of *Fis1* gene expression in skeletal muscles (mean ± SD)
Abbreviations: ET: Endurance training; HIIT: High-intensity interval training

exercise in the study of Yoo *et al.* [37] In their study, Axelrod *et al.* [38] showed that treadmill aerobic exercise (5 days/week, 12 weeks) did not significantly alter the expression of proteins related to the fusion of human muscles. OPA1 and mitofusins are related to mitochondrial fusion, which plays a pivotal role in muscle [34,39]. Furthermore, there are proteins, such as DRP1 and FIS1 which are involved in damaged mitochondria elimination through mitochondrial fission [39]. We showed that exercise, especially aerobic exercise training, caused a significant increase in the expression of MFN1 protein and *Opa1* gene. Axelrod *et al.* [38] also reported that aerobic exercise (12 weeks) significantly decreased the expression of proteins involved in mitochondrial fission (FIS1 and Parkin). Our data showed that aerobic training significantly increased the expression of DRP1 protein and has no effect on the expression of FIS1. The data of Axelrod *et al.*'s study [38] are not in line with our findings, probably due to the longer duration of exercise in their study and its implementation on human subjects [38]. It has been demonstrated that old mice exhibited increased protein expression of FIS1 and decreased level of MFN2, which

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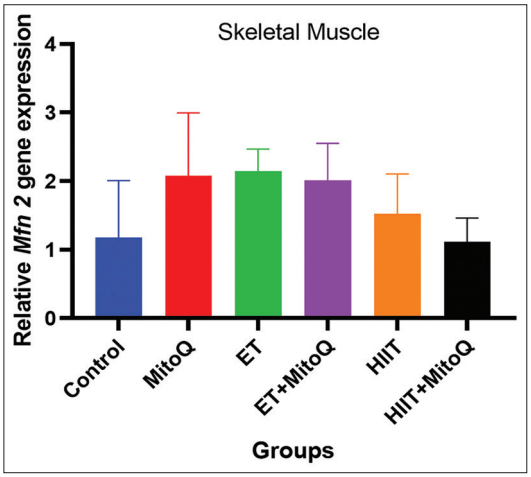

Figure 4. Quantitation of *Mfn2* gene expression in skeletal muscles (mean ± SD)
Abbreviation: ET: Endurance training; HIIT: High-intensity interval training

were reversible by exercise [40]. Ding *et al*. showed that after exercise, mitofusins and FIS1 expression increased in muscle compared to the other groups [33,38]. The results from our and Ding *et al*.'s studies confirm the inducing effect of exercise on mitochondrial fusion, along with the improvement of fission.

MitoQ is a targeted mitochondrial antioxidant that operates primarily by penetrating the mitochondrial membrane and selectively scavenging free radicals, thereby protecting mitochondrial function from oxidative damage. By delivering ubiquinone directly to the mitochondria, MitoQ enhances the electron transport chain's efficiency, leading to improved ATP production and energy metabolism. In addition, it has been shown to modulate mitochondrial dynamics by regulating key regulatory proteins involved in fission and fusion processes, thus maintaining optimal mitochondrial morphology and functionality [16-19]. The interplay between MitoQ and various physiological stimuli, such as exercise training, suggests a role in optimizing mitochondrial health, highlighting its potential as a therapeutic supplement in conditions characterized by mitochondrial dysfunction [20,35,40,41-43]. The MitoQ treatment prevented excessive mitochondrial fragmentation by regulating DRP1 phosphorylation. More importantly, MitoQ maintained aerobic respiration and reduced anaerobic respiration by regulating reprogramming of intracellular energy metabolism, which enhanced cellular ATP production [21]. In the current study, we did not observe any effect of ET alone on the MFN1 protein and *Opa1* gene expression when comparing this independent treatment with the combined ET + MitoQ regimen. On the other hand, we observed an increase in DRP1 protein level in both the ET and ET + MitoQ groups. It has been previously reported that mitochondrial adaptations following muscle exercise are not affected by MitoQ [35]. Furthermore, it has been reported that even though MitoQ augmented PGC-1α expression in muscles, the increase in mitochondrial content caused by exercise remained unaltered by MitoQ treatment [35]. Despite these studies, Williamson *et al*. [41] showed that MitoQ has a protective effect on the mitochondrial genome in muscle tissue post-exercise. In another study on human NP cells, they showed that administration of MitoQ decreased apoptosis and the expression of DRP1 and FIS1 while increasing the expression of proteins involved in fusion [42]. In our study, the administration of MitoQ neutralized the enhancing effects of ET on fusion, and in addition, in the rats that performed HIIT exercise, it enhanced fission by increasing the expression of DRP1 protein (in the HIIT + MitoQ group). These findings contradict the previous studies where MitoQ had no effect in the groups that performed exercise [42]. Given that exercise, particularly aerobic exercise influences the mitochondria and MitoQ specifically targets the mitochondria, the results in the ET + MitoQ and HIIT + MitoQ groups should align more closely with predictions. Despite the contradiction with other studies, it can be concluded that the duration of exercise, the target tissue, the way of MitoQ administration, and the dose of MitoQ are important factors that can affect the results. MitoQ ameliorates mitochondrial dysregulation in heart failure by attenuating hydrogen peroxide generation and increasing mitochondrial respiration [43]. It has also been reported that MitoQ administration reduces mitochondrial damage [44].

Moreover, the apparent discrepancy between our findings and previous studies can be attributed to several factors, including the duration of exercise, the specific muscle tissues examined, and the different methodologies employed for MitoQ administration [35,40]. For instance, previous literature has indicated that aerobic training can enhance mitochondrial function without necessarily altering dynamics [36], yet our study underscores that prolonged and specific exercise interventions, such as ET, can lead to significant changes in protein expression related to both fusion and fission processes [34]. In addition, the dynamic regulation of these proteins, such as DRP1's role in fission and MFN1 and OPA1's roles in fusion, highlights the bidirectional nature of mitochondrial adaptations to exercise [38]. Thus, a comprehensive exploration of how exercise influences mitochondrial dynamics, particularly in combination with specific pharmacological interventions like MitoQ, is essential to deepen our understanding of mitochondrial function in skeletal muscle. Future research should continue to investigate these interactions with varying exercise intensities and durations to elucidate the complexities of mitochondrial dynamics.

Limitations of the current study include the usage of a single MitoQ dose and the relatively short duration of MitoQ treatment. The dose and treatment duration employed in this study were based on the previous studies. In our experience, intraperitoneal injection of MitoQ to rats could be lethal; therefore, we administered it through the drinking water. The toxic effects of this supplement may be related to the role of mitochondria as stress sensors, and it can modulate nuclear functioning through retrograde signaling. Furthermore, another limitation of this research is the housekeeping gene/protein used, *that is*, β-actin. Since this study involves monitoring mitochondrial changes, housekeeping proteins like translocase of the outer membrane 22 or voltage-dependent anion channel and housekeeping gene like mitochondrial processing subunit alpha (*Pmpca*) should be used instead.

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5. Conclusion

The findings of this study highlight the differential impacts of exercise modalities, ET and HIIT, on mitochondrial quality control in skeletal muscle. Our results indicate that ET significantly enhances mitochondrial dynamics, evidenced by a robust increase in the expression levels of proteins linked to mitochondrial fusion and fission, specifically DRP1, MFN1, and OPA1. These adaptations underscore ET's superior capacity to improve mitochondrial function, which is crucial for muscle metabolism and performance. HIIT, while beneficial, primarily promoted mitochondrial fusion as indicated by the increase in MFN1 protein levels, suggesting a more focused impact on mitochondrial dynamics under anaerobic conditions. Interestingly, although MitoQ treatment alone did not elicit substantial changes in mitochondrial dynamics, its combination with exercise training shed light on their complex interactions: in combination with ET, MitoQ appeared to downregulate mitochondrial protein expression, whereas coupled with HIIT, it further enhanced DRP1 expression, suggesting the varying effects of this pharmacological intervention depending on the exercise modality. This finding is critical, as it implies that while MitoQ may support certain mitochondrial functions, its effects are context-dependent and highlight the necessity of investigating specific exercise protocols in conjunction with administration of mitochondria-targeted therapies.

To build a more comprehensive understanding of the mechanisms underlying these observations, further research is necessary. Future studies should explore varying doses and duration of MitoQ treatment to better elucidate its effects on mitochondrial dynamics and function across different exercise intensities. By expanding our examination of these interactions and understanding their implications for skeletal muscle physiology, we can advance the understanding of metabolic control and develop more effective interventions for enhancing mitochondrial health in various populations.

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Conflicts of Interest

The authors declare no competing interest.

Author Contributions

Conceptualization: Beydolah Shahouzehi, Soheil Aminizadeh
Formal analysis: Beydolah Shahouzehi, Soheil Aminizadeh, Hamid Najafipour, Yaser Masoumi-Ardakani
Investigation: Beydolah Shahouzehi, Soheil Aminizadeh, Hamid Najafipour, Yaser Masoumi-Ardakani
Methodology: Beydolah Shahouzehi, Soheil Aminizadeh, Mohammad Pourranjbar
Writing – original draft: All authors
Writing – review & editing: All author

Ethics Approval and Consent to Participate

The animal procedure was approved by the guidelines of the Animal Care Committee of the Ethics Committee of Kerman University of Medical Sciences (IR.KMU.REC.1400.292). All methods were performed in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines 2.0.

Consent for Publication

Not applicable.

Availability of Data

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Karbowski M. Mitochondria on Guard: Role of Mitochondrial Fusion and Fission in the Regulation of Apoptosis. Adv Exp Med Biol 2010;687:131-42. doi: 10.1007/978-1-4419-6706-0_8

[2] Wu T, Li Z, Wei Y. Advances in Understanding Mechanisms Underlying Mitochondrial Structure and Function Damage by Ozone. Sci Total Environ 2023;861:160589. doi: 10.1016/j.scitotenv.2022.160589

[3] Memme JM, Erlich AT, Phukan G, Hood DA. Exercise and Mitochondrial Health. J Physiol 2021;599:803-17. doi: 10.1113/JP278853

[4] Meng Q, Su CH. The Impact of Physical Exercise on Oxidative and Nitrosative Stress: Balancing the Benefits and Risks. Antioxidants (Basel) 2024;13:573. doi: 10.3390/antiox13050573

[5] Scarpulla RC, Vega RB, Kelly DP. Transcriptional integration of mitochondrial biogenesis. Trends Endocrinol Metab 2012;23:459-66. doi: 10.1016/j.tem.2012.06.006

[6] Slavin MB, Khemraj P, Hood DA. Exercise, mitochondrial dysfunction and inflammasomes in skeletal muscle. Biomed J 2024;47:100636. doi: 10.1016/j.bj.2023.100636

[7] Sokolova I. Mitochondrial Adaptations to Variable Environments and their Role in Animals' Stress Tolerance. Integr Comp Biol 2018;58:519-31. doi: 10.1093/icb/icy017

[8] Jenner A, Pena-Blanco A, Salvador-Gallego R, Ugarte-Uribe B, Zollo C, Ganief T, *et al*. Drp1 Interacts Directly with Bax to Induce its Activation and Apoptosis. EMBO J 2022;41:e108587.
doi: 10.15252/embj.2021108587

[9] Bhat S, Chin A, Shirakabe A, Ikeda Y, Ikeda S, Zhai P, *et al*. Recruitment of RNA Polymerase ii to Metabolic Gene Promoters is Inhibited in the Failing Heart Possibly through PGC-1Alpha (Peroxisome Proliferator-activated Receptor-Gamma Coactivator-1Alpha) Dysregulation. Circ Heart Fail 2019;12:e005529.
doi: 10.1161/CIRCHEARTFAILURE.118.005529

[10] Gureev AP, Shaforostova EA, Popov VN. Regulation of Mitochondrial Biogenesis as a Way for Active Longevity: Interaction between the nrf2 and pgc-1alpha Signaling Pathways. Front Genet 2019;10:435.
doi: 10.3389/fgene.2019.00435

[11] Menezes TN, Ramalho LS, Bechara LR, Ferreira JCB. Targeting Mitochondrial Fission-fusion Imbalance in Heart Failure. Curr Tissue Microenviron Rep 2020;1:239-47.

[12] Youle RJ, Van der Bliek AM. Mitochondrial Fission, Fusion, and Stress. Science 2012;337:1062-5.
doi: 10.1126/science.1219855

[13] Hernandez-Resendiz S, Prunier F, Girao H, Dorn G, Hausenloy DJ, Action E-CC. Targeting Mitochondrial Fusion and Fission Proteins for Cardioprotection. J Cell Mol Med 2020;24:6571-85.

[14] Schrepfer E, Scorrano L. Mitofusins, from Mitochondria to Metabolism. Mol Cell 2016;61:683-94.
doi: 10.1016/j.molcel.2016.02.022

[15] Filadi R, Pendin D, Pizzo P. Mitofusin 2: From Functions to Disease. Cell Death Dis 2018;9:330.
doi: 10.1038/s41419-017-0023-6

[16] Ihenacho UK, Meacham KA, Harwig MC, Widlansky ME, Hill RB. Mitochondrial Fission Protein 1: Emerging Roles in Organellar form and Function in Health and Disease. Front Endocrinol (Lausanne) 2021;12:660095.
doi: 10.3389/fendo.2021.660095

[17] Mao P, Manczak M, Shirendeb UP, Reddy PH. MitoQ, a Mitochondria-targeted Antioxidant, Delays Disease Progression and Alleviates Pathogenesis in an Experimental Autoimmune Encephalomyelitis Mouse Model of Multiple Sclerosis. Biochim Biophys Acta 2013;1832:2322-31.
doi: 10.1016/j.bbadis.2013.09.005

[18] Tauskela JS. MitoQ--a Mitochondria-Targeted Antioxidant. IDrugs 2007;10:399-412.

[19] Pham T, MacRae CL, Broome SC, D'Souza RF, Narang R, Wang HW, *et al*. MitoQ and CoQ10 Supplementation Mildly Suppresses Skeletal Muscle Mitochondrial Hydrogen Peroxide Levels without Impacting Mitochondrial Function in Middle-aged Men. Eur J Appl Physiol 2020;120:1657-9.

[20] Sulaimon LA, Afolabi LO, Adisa RA, Ayankojo AG, Afolabi MO, Adewolu AM, *et al*. Pharmacological Significance of MitoQ in Ameliorating Mitochondria-Related Diseases. Adv Redox Res 2022;5:100037.
doi: 10.1016/j.arres.2022.100037

[21] Tsui KH, Li CJ. Mitoquinone Shifts Energy Metabolism to Reduce ROS-induced oxeiptosis in female granulosa cells and mouse oocytes. Aging (Albany NY) 2023;15:246-60.
doi: 10.18632/aging.204475

[22] Chen S, Wang Y, Zhang H, Chen R, Lv F, Li Z, *et al*. The Antioxidant MitoQ Protects Against CSE-induced Endothelial Barrier Injury and Inflammation by Inhibiting ROS and Autophagy in Human Umbilical Vein Endothelial Cells. Int J Biol Sci 2019;15:1440-51.
doi: 10.7150/ijbs.30193

[23] Merry TL, Ristow M. Do Antioxidant Supplements Interfere with Skeletal Muscle Adaptation to Exercise training? J Physiol 2016;594:5135-47.
doi: 10.1113/JP270654

[24] Moore TM, Zhou Z, Cohn W, Norheim F, Lin AJ, Kalajian N, *et al*. The impact of exercise on mitochondrial dynamics and the role of drp1 in exercise performance and training adaptations in skeletal muscle. Mol Metab 2019;21:51-67.
doi: 10.1016/j.molmet.2018.11.012

[25] Braakhuis AJ, Nagulan R, Somerville V. The Effect of MitoQ on Aging-related Biomarkers: A Systematic Review and Meta-analysis. Oxid Med Cell Longev 2018;2018:8575263.
doi: 10.1155/2018/8575263

[26] Zadeh HJ, Roholamini Z, Aminizadeh S, Deh-Ahmadi MA. Endurance Training and MitoQ Supplementation Improve Spatial Memory, VEGF Expression, and Neurogenic Factors in Hippocampal Tissue of Rats. J Clin Transl Res 2023;9:1-7.

[27] Hu J, Cai M, Shang Q, Li Z, Feng Y, Liu B, *et al*. Elevated Lactate by High-Intensity Interval Training Regulates the Hippocampal Bdnf Expression and the Mitochondrial Quality Control System. Front Physiol 2021;12:629914.
doi: 10.3389/fphys.2021.629914

[28] Verboven M, Cuypers A, Deluyker D, Lambrichts I, Eijnde BO, Hansen D, *et al*. High Intensity Training Improves Cardiac Function in Healthy Rats. Sci Rep 2019;9:5612.
doi: 10.1038/s41598-019-42023-1

[29] Batacan RB Jr., Duncan MJ, Dalbo VJ, Connolly KJ, Fenning AS. Light-intensity and High-intensity Interval Training Improve Cardiometabolic Health in Rats. Appl Physiol Nutr Metab 2016;41:945-52.
doi: 10.1139/apnm-2016-0037

[30] Pour MB, Joukar S, Hovanloo F, Najafipour H. Long-term Low-intensity Endurance Exercise Along with Blood-flow Restriction Improves Muscle Mass and Neuromuscular Junction Compartments in Old Rats. Iran J Med Sci 2017;42:569-576.

[31] Mohammadi A, Fallah H, Shahouzehi B, Najafipour H. Effect of LXR Agonist t0901317 and MIR-33inhibitor on SIRT1-AMPK and Circulating HDL-C levels. Bulg Chem Commun 2018;50:111-8.

[32] Jiang HK, Wang YH, Sun L, He X, Zhao M, Feng ZH, *et al*. Aerobic interval Training Attenuates Mitochondrial Dysfunction in Rats Post-myocardial Infarction: Roles of Mitochondrial Network Dynamics. Int J Mol Sci 2014;15:5304-22.
doi: 10.3390/ijms15045304

[33] Ding H, Jiang N, Liu H, Liu X, Liu D, Zhao F, *et al*. Response of mitochondrial Fusion and Fission Protein Gene Expression to Exercise in rat skeletal muscle. Biochim Biophys Acta 2010;1800:250-6.
doi: 10.1016/j.bbagen.2009.08.007

[34] Hood DA, Memme JM, Oliveira AN, Triolo M. Maintenance of skeletal muscle mitochondria in health, exercise, and aging. Annu Rev Physiol 2019;81:19-41.
doi: 10.1146/annurev-physiol-020518-114310

[35] Broome SC, Pham T, Braakhuis AJ, Narang R, Wang HW, Hickey AJR, *et al*. MitoQ Supplementation Augments Acute Exercise-induced Increases in Muscle pgc1α mRNA and Improves Training-induced Increases in Peak Power Independent of Mitochondrial Content and Function in Untrained Middle-aged Men. Redox Biol 2022;53:102341.
doi: 10.1016/j.redox.2022.102341

[36] Lee YJ, Jeong SY, Karbowski M, Smith CL, Youle RJ. Roles of the Mammalian Mitochondrial Fission and Fusion Mediators FIS1, DRP1, and OPA1 in Apoptosis. Mol Biol Cell 2004;15:5001-11.
doi: 10.1091/mbc.e04-04-0294

[37] Yoo SZ, No MH, Heo JW, Park DH, Kang JH, Kim JH, *et al*. Effects of Acute Exercise on Mitochondrial Function, Dynamics, and Mitophagy in Rat Cardiac and Skeletal Muscles. Int Neurourol J 2019;23:S22-31.
doi: 10.5213/inj.1938038.019

[38] Axelrod CL, Fealy CE, Mulya A, Kirwan JP. Exercise Training Remodels Human Skeletal Muscle Mitochondrial Fission and Fusion Machinery Towards a Pro-Elongation Phenotype. Acta Physiol (Oxf) 2019;225:e13216.
doi: 10.1111/apha.13216

[39] Hall AR, Burke N, Dongworth RK, Hausenloy DJ. Mitochondrial fusion and Fission Proteins: Novel Therapeutic Targets for Combating Cardiovascular disease. Br J Pharmacol 2014;171:1890-906.
doi: 10.1111/bph.12516

[40] Gioscia-Ryan RA, Battson ML, Cuevas LM, Zigler MC, Sindler AL, Seals DR. Voluntary Aerobic Exercise Increases Arterial Resilience and Mitochondrial Health with Aging in Mice. Aging (Albany NY) 2016;8:2897-914.
doi: 10.18632/aging.101099

[41] Williamson J, Hughes CM, Cobley JN, Davison GW. The Mitochondria-targeted Antioxidant MitoQ, Attenuates Exercise-induced Mitochondrial DNA Damage. Redox Biol 2020;36:101673.
doi: 10.1016/j.redox.2020.101673

[42] Kang L, Liu S, Li J, Tian Y, Xue Y, Liu X. The Mitochondria-targeted Anti-Oxidant MitoQ Protects Against Intervertebral Disc Degeneration by Ameliorating Mitochondrial Dysfunction and Redox Imbalance. Cell Prolif 2020;53:e12779.
doi: 10.1111/cpr.12779

[43] Ribeiro Junior RF, Dabkowski ER, Shekar KC, Connell KAO, Hecker PA, Murphy MP. MitoQ Improves Mitochondrial Dysfunction in Heart Failure Induced by Pressure Overload. Free Radic Biol Med 2018;117:18-29.
doi: 10.1016/j.freeradbiomed.2018.01.012

[44] Lowes DA, Webster NR, Murphy MP, Galley HF. Antioxidants that Protect Mitochondria Reduce Interleukin-6 and Oxidative Stress, Improve Mitochondrial Function, and Reduce Biochemical Markers of Organ Dysfunction in a Rat Model of Acute Sepsis. Br J Anaesth 2013;110:472-80.
doi: 10.1093/bja/aes577