Research Article

Six-week combined exercise modulates mitochondrial dynamics (MFN1/DRP1) and oxidative stress (MDA/SOD) in fast- and slow-twitch muscles of aged rats

Sina Gholaminezhad Kolachahi[1*], Farhad Rahmani-nia[2], Mohammad Reza Fadaei Chafy[3]

Abstract

Aging is associated with mitochondrial dysfunction, which leads to decreased cellular function and the development of age-related diseases. Exercise training is considered one of the most effective strategies for improving muscle cell function. The aim of the present study was to investigate the effect of six-week combined exercise on mitochondrial dynamics and biogenesis markers (MFN1, DRP1) as well as oxidative stress markers (MDA and SOD) in fast- and slow-twitch muscles of aged rats. In this study, 16 male Wistar rats (463.2 ± 9.3 g) were randomly divided into two groups (n=8 per group): control and resistance-endurance training. The training group underwent combined resistance-endurance training, 6 days a week for 6 weeks (3 resistance days, 3 endurance days). Forty-eight hours after the last training session, animals were sacrificed and fast-twitch (gastrocnemius) and slow-twitch (soleus) muscle tissues were collected. Gene expression levels of mitofusin 1 (MFN1), dynamin-related protein 1 (DRP1) were measured by real-time PCR (RT-PCR). In slow-twitch muscle, exercise training significantly increased mRNA expression levels of SOD genes, and significantly decreased mRNA expression of DRP1 and the concentration of MDA compared to the control group ($p<0.05$). Similarly, in fast-twitch muscle, six weeks of combined training significantly increased SOD gene expressions and decreased DRP1 mRNA and MDA levels compared to controls ($p<0.05$). Combined exercise training positively modulates mitochondrial biogenesis and dynamics markers (decreased DRP1 mRNA) and enhances antioxidant capacity (increased SOD gene expression and enzyme activity, decreased MDA levels) in both fast- and slow-twitch muscles of aged rats, highlighting its significant role in mitigating age-associated mitochondrial dysfunction. These findings reflect improvements in markers of mitochondrial quality control and oxidative stress rather than direct measurements of mitochondrial function.

Key Words: Aging, Mitochondrial dynamics, Oxidative stress, Combined exercise, Fast-twitch muscle, Slow-twitch muscle

1. Department of Exercise Physiology, University Campus2, University of Guilan, Rasht, Guilan, Iran. 2. Professor of Exercise Physiology, Faculty of Physical Education and Sport Sciences, University of Guilan, Rasht, Iran. 3. Department of Physical Education and Sport Science, Ra.C., Islamic Azad University, Rasht, Iran.

*Author for correspondence: phd.sina2022@gmail.com

S G K: 0000-0003-3400-9372; F R: 0000-0001-7021-0060; MR F C: 0000-0003-0265-5329

Received 16 March 2025; Accepted 30 June 2025

Introduction

Sarcopenia affects people from about the fourth decade of life and is associated with a 30–50% decrease in skeletal muscle mass and function by the time people reach approximately 80 years of age (Cruz-Jentoft & Sayer, 2019). At the cellular level, aging can affect the function and properties of skeletal muscle fibers. However, both fast-twitch and slow-twitch muscle fibers are affected by sarcopenia at the cellular level. Biochemical and metabolic changes also occur in muscle with age. Mitochondrial DNA mutations have been reported following oxidative damage and decreased mitochondrial protein synthesis in aging and are likely associated with decreased glycolytic and oxidative enzyme activity, muscle cell creatine phosphate and ATP stores, mitochondrial volume, and a modest decrease in total body metabolic capacity (Chen et al., 2023). These metabolic changes in muscle impair the overall fitness capacity of older adults, a major component of the approximately 30% decline in the ability to utilize VO2max during exercise (Radak et al., 2019). Early studies in older adults have also shown that aging is associated with a decrease in muscle protein synthesis, which may be responsible for the gradual decline in muscle mass (Musci et al., 2025; Zhong et al., 2023).

Mitochondrial content and function are highly malleable in response to changes in metabolic energy demand, which has led to a surge in mitochondrial research over the past decade. Exercise is a well-established stimulus for increasing mitochondrial function in skeletal muscle. However, a decline in mitochondrial function can also occur, forcing the muscle to derive a greater proportion of its energy from the glycolytic pathway due to increased energy demand. Independent of inactivity, there is evidence that mitochondria decline with age and in a variety of conditions, including mitochondrial DNA (mtDNA) diseases, lysosomal storage defects in cancer, and in the absence of key regulatory proteins, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), p53, and SirT1 (Sinha et al., 2024).

Mitochondria are present in an interconnected network in

skeletal muscle, known as the mitochondrial reticulum (Glancy et al., 2015). This makes sense because mitochondria are highly mobile organelles that continuously undergo fusion and fission events to regulate the organization of the network within the cell (Iqbal & Hood, 2014). Cytochrome c oxidase (COX), the last enzyme in the mitochondrial electron transport chain that drives oxidative phosphorylation, is also affected by fusion and fission. Fusion refers to the recruitment of relatively new mitochondrial fragments into the network and is mediated by GTPases, Mfn1/2, which connect the outer mitochondrial membranes (OM) and Opa1, which is primarily responsible for inner membrane fusion (Kamerkar et al., 2025). Fusion contributes to the expansion and elongation of the mitochondrial reticulum, and more complex and extensive morphologies are characteristic of healthy and trained individuals (Zhao & Gao, 2024). This process (fusion) is associated with greater metabolic flexibility, lipid metabolism, and the widespread distribution of mtDNA and metabolites in muscle cells. Conversely, fission refers to the removal of parts of the network and is controlled by the dynamin-associated GTPase Drp1 and its receptor Fis1 (Preminger & Schuldiner, 2024). Fission events are critical for maintaining a healthy network, as this process can selectively remove parts of the network that have become dysfunctional and help limit the release of toxic byproducts such as ROS or mtDNA mutations. However, overreliance on fission can lead to small, fragmented mitochondria that have been shown to produce more ROS and less adenosine triphosphate (ATP). Thus, mitochondrial morphology is intrinsically linked to mitochondrial function and quality. Aging and exercise training are two reversible processes that affect mitochondrial morphology. However, exercise training is one of the important factors that plays a role in controlling muscle atrophy and age-related muscle mitochondrial quality.

Several studies have shown that aerobic exercise improves VO2max, mitochondrial density and activity, insulin sensitivity, and energy expenditure in young and elderly subjects (Mahatme et al., 2022; Yeo et al., 2022). Prolonged and intense aerobic exercise has been reported to increase muscle protein synthesis in human subjects (Phillips et al., 2009; Wilkinson et al., 2008). Additionally, Sheffield-Moore et al. (2004) demonstrated that older men have a similar muscle protein synthesis response to aerobic exercise compared with younger men, indicating preserved anabolic responsiveness despite aging (Sheffield-Moore et al., 2004). Although aerobic exercise does not induce overt muscle hypertrophy, some studies have shown that intense aerobic exercise can induce some degree of hypertrophy, as confirmed by increases in calf circumference, muscle fiber area, and satellite cell activation (Kaczmarek et al., 2021; Thomas et al., 2022). Since limited studies have examined changes in the antioxidant system and mitochondrial dynamics (MFN1/DRP1) in various muscle tissues of the elderly, therefore, we hypothesized that six weeks of combined exercise would modulate mitochondrial dynamics (MFN1/DRP1) and oxidative stress (MDA/SOD) in fast- and slow-twitch muscles of aged rats.

Materials and methods

Animals

In the present study, 16 male Wistar rats were obtained from the Pasteur Institute of Iran at the age of 24 months (463.2 ± 9.3). The inclusion criteria for the study included being male, elderly, within the desired weight range, and in good health, and no previous use of any medication. The exclusion criteria also included the use of analgesic and anti-toxic drugs, having any disease or inflammation, and the weight of the rat not being appropriate for the present study. The animals were maintained under standard laboratory conditions with temperature (22 ± 3 degrees Celsius) and humidity (about 45%) in a 12-hour light-dark cycle until the end of the experiments and exercise training period. (Lighting time was from 6:00 AM to 6:00 PM). The purpose of establishing this cycle was to create a natural living condition for the mice. All animals had free access to water and standard food for laboratory animals. It should be noted that all ethical principles of the present study were observed in accordance with the principles of working with laboratory animals approved by the University of Guilan (Guilan, Iran), and all its steps were approved by the ethics committee of that university with the ethics code IR.GUILAN.REC.1404.063. After one week of familiarization with the laboratory environment, the animals were divided into two groups: control (8 rats) and resistance-endurance training (8 rats).

Resistance-endurance training program

The training program was carried out for 6 weeks as a combination of resistance training on a ladder and aerobic training on an animal treadmill (Tehiz Gostar Iranian, model 2016). Resistance training consisted of 3 training sessions per week (Saturday, Monday, Wednesday) for 6 weeks, each session consisting of 3 sets and each set consisting of 4 times climbing a special ladder with a height of 1 meter and 26 steps with a distance of 4 cm between the steps. A 30-second rest was provided for the animals between each set. After tying a weight to the animals' tails, they were forced to climb the vertical ladder. The principle of overloading was carried out by increasing the percentage of body weight on a weekly basis, so that in the first week the amount of weight tied to the animal's tail was 30%, and gradually from the second week it was 70%, the third week 100%, the fourth week 120%, the fifth week 140% and the sixth week 160% of their body weight (Kim et al., 2015). The weight increase from 30% in week 1 to 70% in week 2 was achieved gradually within week 2 by small daily increments to allow the animals to adapt comfortably. Specifically, the weight was increased in equal steps each training day of week 2 rather than a single jump

at the start of the week. Similarly, for the subsequent weeks, the weekly percentage reflects the target weight load for that week, maintained constant across all training sessions of that week.

Blood collection and tissue sampling

48 hours after the last intervention, all rats were fasted for 8-10 hours and weighed before starting tissue sampling. After weighing, the animals were anesthetized with intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). After complete anesthesia and pain testing and ensuring unconsciousness, blood was collected from the left ventricle of the heart (Figure 3-5). Then, fast-twitch (gastrocnemius) and slow-twitch (soleus) muscle tissue were quickly removed and washed with physiological serum to remove blood and excess material, and the tissue was placed in a 2-ml coded microtube. The microtube was transferred to a nitrogen tank, then stored in a -80°C freezer until cellular analyses.

Enzyme assay

To lyse tissue samples, diluted buffer (Lysing Buffer) was used and 500 microliters of buffer were added per 100 mg of tissue sample and homogenized. Then, the desired sample was centrifuged at 12,000 rpm for 5 minutes at 4°C and the supernatant was used as a sample. The prepared sample was analyzed for SOD (superoxide dismutase), MDA (malondialdehyde) levels according to the instructions of the kits of Navand Health Iran Company and the spectrophotometric method.

Gene expression at skeletal muscle

For molecular investigations at the level of gene expression (MFN1/DRP1), RNA extraction was first performed from the tissue in all the studied groups, according to the manufacturer's protocol (Qiagen, Germany). For this purpose, 200 µL Trizol reagent was added to the samples and incubated at -80°C for 24 hours. The tissue samples were homogenized thoroughly in the semi-frozen state using a pestle (or bead beater) to ensure complete lysis before proceeding. Then, 100 µL chloroform was added to each homogenized sample and mixed vigorously for 1 minute. The resulting solution was centrifuged at 12,000 rpm for 10 minutes at 4°C. The clear aqueous phase on top containing the RNA was carefully removed and transferred into a DEPC-treated microtube. Subsequently, 1 mL of isopropanol was added and mixed by inversion for 1 minute to precipitate the RNA. The samples were centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatant was discarded, and the RNA pellet was washed with 1 mL of 70% ethanol, vortexed and centrifuged for 10 minutes at 7,500 rpm at 4°C. The supernatant was discarded, and the RNA pellet was air-dried inside the tube. Then, 20 µL DEPC-treated distilled water preheated at 60°C was added to dissolve the RNA pellet, and the samples were incubated at 60°C for 5 minutes.

After confirming RNA purity and concentration by spectrophotometry, cDNA synthesis was performed using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Catalogue No. K1622) following the manufacturer's instructions. The synthesized cDNA was then used for quantitative real-time PCR (qPCR).

Measurement of skeletal muscle expression levels of MFN1 and DRP1 genes was carried out by qPCR using a StepOnePlus Real-Time PCR System (Applied Biosystems). The qPCR reactions were prepared in a final volume of 20 µL, containing cDNA template, specific primers, and SYBR Green master mix. Cycling conditions were: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec, annealing at 58°C for 30 sec, and extension at 72°C for 30 sec. Primer efficiencies were validated by standard curves with serial cDNA dilutions, and efficiencies were between 90-105%. The sequences of primers used are reported in Table 1.

Glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) was used as the housekeeping gene. Expression stability of GAPDH was confirmed by demonstrating no significant differences in Ct values between groups (average Ct = 18.5 ± 0.7, $p > 0.05$).

Relative gene expression was calculated using the ΔΔCt method with the formula:

Fold Change = 2^(-ΔΔCt), where

ΔCt = Ct_Target gene - Ct_Housekeeping gene, and

ΔΔCt = ΔCt_Sample - ΔCt_Reference sample (control).

Statistical analysis

The normality of data distribution was checked and confirmed using the Shapiro-Wilk test. In order to determine the significance of the difference between the variables of the research groups, one-way analysis of variance and post hoc Tukey's test were used. Mean and standard deviation were used for descriptive data reporting. After collecting the required information, it was analyzed using SPSS version 26 statistical software at a significance level of at least $p<0.05$.

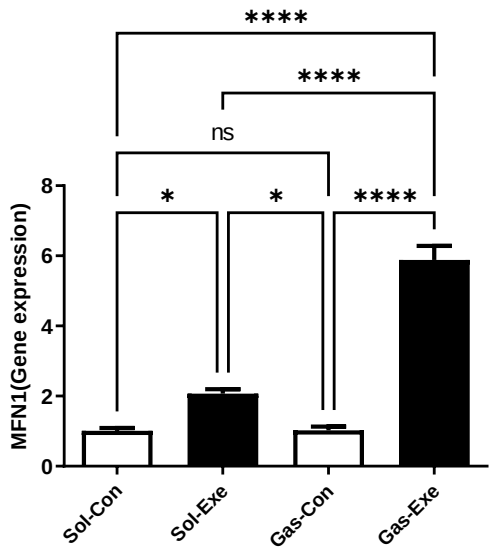
Table 1. The genes primer sequences.

Genes	Primer sequence
MFN1	F: 5'- ATC GGA TCT TTT TTG TTT CAG C -3' R: 5'- CTC CTG TAA TCT TGC CTG -3'
DRP1	F: 5'- ATGGATGTATTGATGGGAAGG -3' R: 5'- TTCTGTTGGCCAGAGATGGGT -3'
GAPDH	F: 5' CCCTGTTGCTGTAGGCGTATT 3' R: 5' TGACATCAAGAAGGTGGTGAA 3'

Results

The changes in the expression of DRP1 and MFN1 genes are shown in Fig. 1 (Fig. 1, A, B). According to the results of one-way ANOVA, there was a significant difference in the expression of DRP1 (F=20.88, p=0.0001) and MFN1 (F=113.1, p=0.0001) genes in different groups.

A)



B)

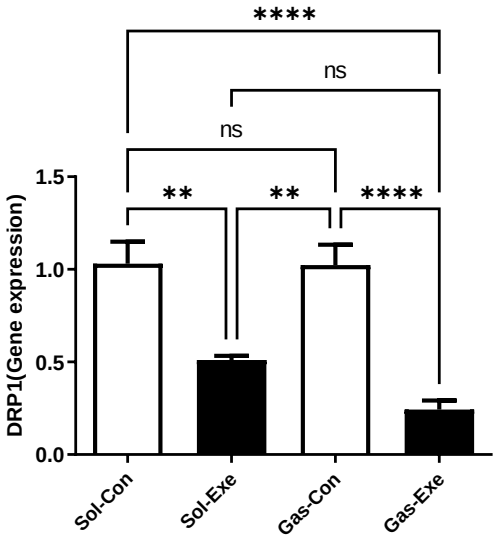


Figure 1. MFN1 (A) and DRP 1 (B) gene expression in gastrocnemius and soleus muscle fibers of different groups of aged rats following 6 weeks of combined resistance-endurance training. Data are presented as mean and standard deviation. The significance level is ($p \geq 0.05$). *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001. Abbreviations: Sol-Con: soleus-control, Sol-Exe: soleus-exercise, Gas-Con: gastrocnemius-control, Gas-Exe: gastrocnemius-exercise

In the soleus muscle, it was found that combined training significantly increased the expression of MFN1 gene compared to the control group (p<0.0001). In the gastrocnemius muscle, 6 weeks of combined training also increased the expression of MFN1 gene compared to the control group (p<0.0001). In the comparison between the exercise groups in both muscles, it was found that there was no significant difference in the expression of MFN1 gene in the gastrocnemius muscle and soleus muscle (p=0.994).

In the soleus muscle, it was found that exercise significantly reduced DRP1 gene expression levels compared to the control group (p=0.0028). In the gastrocnemius muscle, 6 weeks of combined training also reduced DRP1 gene expression levels compared to the control group (p<0.0001). In the comparison between the exercise groups in both muscles, it was found that there was no significant difference in DRP1 gene expression in the gastrocnemius and soleus muscles (p=0.1631).

Changes in oxidative enzymes are also shown in Fig. 2 (Fig. 2 A, B). Based on the results, MDA levels in the exercise groups showed a significant decrease in both muscles compared to the control (p<0.05). The greatest decrease in MDA was in the gastrocnemius muscle exercise group (Figure 2A).

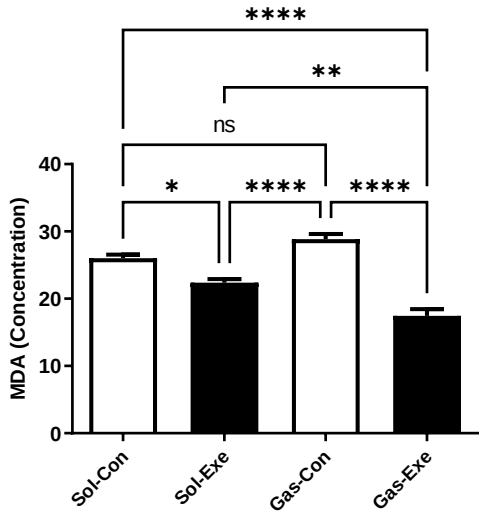
In the soleus muscle, it was found that exercise significantly increased the levels of SOD enzyme compared to the control group (p=0.0002). In the gastrocnemius muscle, 6 weeks of combined training also increased the levels of SOD enzyme compared to the control group (p<0.0001). In the comparison between the exercise groups in both muscles, it was found that the SOD enzyme in the gastrocnemius muscle did not change significantly compared to the soleus muscle (p=0.1449).

Discussion

Effective control of mitochondrial biogenesis and turnover is critical for maintaining energy production, preventing endogenous oxidative stress, and promoting healthy aging (Qin et al., 2024). Dysfunction of mitochondrial biogenesis or fission/fusion, as well as disruption of the balance of mitochondrial oxidant and antioxidant enzymes, has been linked to neuromuscular diseases and aging, and a better understanding of the regulation of these processes should help us ultimately control the aging process. Exercise training can also regulate (negatively or positively) some mitochondrial-related gene factors by inducing mitochondrial biogenesis and creating adaptations at the cellular level. Therefore, the aim of the present study was to investigate the effect of six-week combined exercise on mitochondrial dynamics (MFN1/DRP1) and oxidative stress (MDA/SOD) in fast- and slow-twitch muscles of aged rats.

In the soleus muscle, it was found that training significantly increased the expression levels of the MFN1 gene compared to

A)



B)

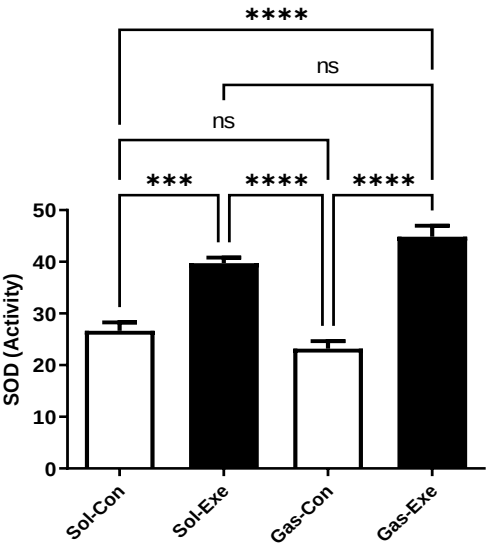


Figure 2. Changes in MDA and SOD enzymes in gastrocnemius and soleus muscle fibers of different groups of aged rats following 6 weeks of combined resistance-endurance training. Data are shown as mean and standard deviation. The significance level is ($p \geq 0.05$). *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ****: $p<0.0001$. Abbreviations: Sol-Con: soleus muscle-control, Sol-Exe: soleus muscle-exercise, Gas-Con: gastrocnemius muscle-control, Gas-Exe: gastrocnemius muscle-exercise.

the elderly control group (p=0.0158). In the gastrocnemius muscle, 6 weeks of combined training also increased the expression levels of the MFN1 gene compared to the healthy control group (p<0.0001). In the comparison between the training groups in both muscles, it was found that the expression of the MFN1 gene in the gastrocnemius muscle increased significantly compared to the soleus muscle (p<0.0001). Based on these findings, aging is associated with lower levels of MFN1, which the present training modality was successful in positively regulating. The aging process involves a gradual deterioration of metabolic function, accompanied by an increase in the incidence of muscle and metabolic disorders, such as sarcopenia. Whole body oxidative capacity is closely related to skeletal muscle oxidative capacity, and accordingly, several studies have reported that skeletal muscle oxidative capacity decreases during aging (Johnson et al., 2013). The content of several key mitochondrial enzymes has been shown to be reduced in aged compared with young muscle (Ringholm et al., 2023). Furthermore, it has been reported that skeletal muscle respiratory capacity per mitochondrial volume is lower in aged than in young individuals (Larsen et al., 2012). Thus, the age-related decline in mitochondrial transcription factors is a cellular injury that may underlie muscle atrophy and several age-related diseases. Regarding the importance of the balanced regulation of mitochondrial dynamics, which is influenced by MFN1 and other mitochondrial transcription factors, studies have shown that mice with tissue-specific deletion of MFN are insulin-resistant to glucose and show mitochondrial fragmentation and higher content of hydrogen peroxide in skeletal muscle (Sebastián et al., 2012). Based on these findings, the maintenance of mitochondrial structural dynamics is crucial for maintaining muscle metabolic function. In other words, mitochondrial dynamics, particularly the processes of fusion and fission, play a critical role in cellular health and are significantly affected by aging. MFN1 (Mitofusin 1) is a key protein involved in mitochondrial fusion, and as noted, its expression and function are altered during the aging process. Research suggests that aging is associated with changes in MFN1 expression levels. For example, in skeletal muscle, there is a significant increase in MFN1 mRNA levels at early ages (1–3 months of age) in mice, suggesting a potential role for this protein in early mitochondrial adaptations (Bečanović et al., 2021). However, as organisms age, overall mitochondrial function declines, which includes changes in the dynamics controlled by proteins such as MFN1.

In the soleus muscle, it was found that training significantly reduced DRP1 gene expression levels compared to the control group (p=0.0028). In the gastrocnemius muscle, 6 weeks of combined training also reduced DRP1 gene expression levels compared to the healthy control group (p<0.0001). In a comparison between the training groups in both muscles, it was found that there was no significant difference in DRP1 gene expression in the gastrocnemius and soleus muscles (p=0.1631). In a study by Moore et al. (2019), they investigated the effect of acute and chronic exercise on mitochondrial dynamic signaling, including the mitochondrial fission regulator Dynamin-related protein (Drp) 1, on exercise performance and muscle adaptation to exercise. In this study, heterozygous mice as well as healthy and hyperglycemic men were used. Exercise training was also

performed acutely and chronically. Their results showed that endurance exercise affects all aspects of the mitochondrial life cycle, such as fission-fusion, biogenesis, and mitophagy. In this study, Drp1 phosphorylation was significantly increased with acute exercise and decreased to baseline levels during post-exercise recovery. Dnm1L expression was strongly associated with transcripts known to regulate mitochondrial metabolism and exercise adaptation (Moore et al., 2019). It should be noted that the present study measured total DRP1 gene expression by RT-PCR and did not assess DRP1 phosphorylation status, which is an important distinction when comparing acute versus chronic exercise effects on DRP1 signaling. The present study was also a chronic study, with DRP1 changes assessed after 6 weeks. It seems that acute and chronic DRP1 responses to exercise training could be among the reasons for the differences in the results of the present study with those of Moore et al. In a study of mitochondrial DRP1 in aging, Gusdon et al. (2017) also showed that exercise training increases mitochondrial complex I activity and DRP1 expression in the brain of aged mice (Gusdon et al., 2017). This difference in tissue type (brain vs skeletal muscle) likely explains some discrepancies with the present results, highlighting the importance of tissue-specific responses to exercise in aging. Studies with obesity and insulin resistance models have shown that exercise training improves substrate metabolism and insulin sensitivity by reducing the activation of the mitochondrial fission protein Drp1 (Fealy et al., 2014). These results were consistent with the results of the present study, and therefore it seems that in conditions of aging similar to obesity, exercise training with negative regulation of DRP1 has more appropriate effects, which also requires further studies in this field.

Exercise activates various signaling pathways, including those related to SIRT1 and PGC-1α, which are critical for enhancing mitochondrial biogenesis and antioxidant defense. These pathways help to improve the expression of antioxidant enzymes such as SOD and increase GSH synthesis in skeletal muscle. Regular physical activity can also reduce age-related inflammation that contributes to oxidative stress. By reducing inflammatory cytokines, exercise helps to create a more favorable environment for mitochondrial function and health (Li et al., 2022). In summary, combined exercise effectively reduces oxidative stress markers such as MDA while increasing antioxidant enzyme activity (SOD and GSH) in aged mouse muscle. These changes indicate positive effects on mitochondrial biogenesis and the balance of mitochondrial fusion-fission processes and oxidative stress, rather than direct measures of mitochondrial function. These changes contribute to improved mitochondrial function and overall muscle health, highlighting the importance of physical activity in reducing age-related decline in muscle function and metabolic health.

Conclusion

In conclusion, this study demonstrates that a six-week combined exercise regimen effectively modulates mitochondrial dynamics and reduces oxidative stress in the skeletal muscles of aged rats. Specifically, training significantly upregulated MFN1 (promoting mitochondrial fusion) and downregulated DRP1 (reducing excessive fission) in both soleus and gastrocnemius muscles, while simultaneously decreasing the oxidative stress marker MDA and enhancing antioxidant enzyme activity (SOD/GSH). These exercise-induced adaptations counteracted age-associated declines in mitochondrial dynamics regulators and oxidative balance, which are critically linked to metabolic dysfunction and muscle deterioration (e.g., sarcopenia). The findings underscore that combined exercise training is a potent intervention to improve mitochondrial health, enhance antioxidant defenses, and mitigate age-related skeletal muscle decline, highlighting its therapeutic potential for promoting healthy aging.

What is already known on this subject?

Several studies have shown that aerobic exercise improves VO2max, mitochondrial density and activity, insulin sensitivity, and energy expenditure in young and elderly subjects.

What this study adds?

Six-week combined exercise regimen effectively modulates mitochondrial dynamics and reduces oxidative stress in the skeletal muscles of aged rats.

Organ Cross-Talk Tips:

- Both fast-twitch and slow-twitch muscles show similar adaptive responses to combined resistance-endurance exercise, highlighting an intramuscular crosstalk mechanism where exercise training coordinates mitochondrial fusion-fission balance and antioxidant defense to counteract age-related mitochondrial dysfunction and oxidative damage across different muscle fiber types.
- Exercise promotes systemic benefits by enabling muscle to communicate with other organs, supporting healthier aging through mitochondrial and oxidative stress regulation.

Acknowledgements

None

Funding

No sources of funding were sought or awarded for this study.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All ethical principles of the present study were observed in accordance with the principles of working with laboratory animals approved by the University of Guilan (Guilan, Iran), and all its steps were approved by the ethics committee of that university with the ethics code IR.GUILAN.REC.1404.063.

Informed consent Animal study.

Author contributions

Conceptualization: S.G.K, F.R, MR.F.C,; Methodology: S.G.K, F.R, MR.F.C,; Software: S.G.K, F.R, MR.F.C,; Validation: S.G.K, F.R, MR.F.C,; Formal analysis: S.G.K, F.R, MR.F.C,; Investigation: S.G.K, F.R, MR.F.C,; Resources: S.G.K, F.R, MR.F.C,; Data curation: S.G.K, F.R, MR.F.C,; Writing - original draft: S.G.K, F.R, MR.F.C,; Writing - review & editing: S.G.K, F.R, MR.F.C,; Visualization: F.R,; Supervision: S.G.K,; Project administration: MR.F.C,; Funding acquisition: S.G.K.

References

Bečanović, K., Asghar, M., Gadawska, I., Sachdeva, S., Walker, D., Lazarowski, E. R., . . . Leavitt, B. R. (2021). Age-related mitochondrial alterations in brain and skeletal muscle of the YAC128 model of Huntington disease. NPJ aging and mechanisms of disease, 7(1), 26. doi: https://doi.org/10.1038/s41514-021-00079-2

Chen, X., Ji, Y., Liu, R., Zhu, X., Wang, K., Yang, X., . . . Shen, Y. (2023). Mitochondrial dysfunction: roles in skeletal muscle atrophy. Journal of Translational Medicine, 21(1), 503. doi: https://doi.org/10.1186/s12967-023-04369-z

Cruz-Jentoft, A. J., & Sayer, A. A. (2019). Sarcopenia. The lancet, 393(10191), 2636-2646. URL: https://www.thelancet.com/article/S0140-6736(19)31138-9/abstract

Fealy, C. E., Mulya, A., Lai, N., & Kirwan, J. P. (2014). Exercise training decreases activation of the mitochondrial fission protein dynamin-related protein-1 in insulin-resistant human skeletal muscle. Journal of applied physiology, 117(3), 239-245. doi: https://doi.org/10.1152/japplphysiol.01064.2013

Glancy, B., Hartnell, L. M., Malide, D., Yu, Z.-X., Combs, C. A., Connelly, P. S., . . . Balaban, R. S. (2015). Mitochondrial reticulum for cellular energy distribution in muscle. Nature, 523(7562), 617-620. doi: https://doi.org/10.1038/nature14614

Gusdon, A. M., Callio, J., Distefano, G., O'Doherty, R. M., Goodpaster, B. H., Coen, P. M., & Chu, C. T. (2017). Exercise increases mitochondrial complex I activity and DRP1 expression in the brains of aged mice. Experimental gerontology, 90, 1-13. doi: https://doi.org/10.1016/j.exger.2017.01.013

Iqbal, S., & Hood, D. A. (2014). Cytoskeletal regulation of mitochondrial movements in myoblasts. Cytoskeleton, 71(10), 564-572. doi: https://doi.org/10.1002/cm.21188

Johnson, M. L., Robinson, M. M., & Nair, K. S. (2013). Skeletal muscle aging and the mitochondrion. Trends in Endocrinology & Metabolism, 24(5), 247-256. URL: https://www.cell.com/trends/endocrinology-metabolism/abstract/S1043-2760(12)00223-8?large_figure=true

Kaczmarek, A., Kaczmarek, M., Ciałowicz, M., Clemente, F. M., Wolański, P., Badicu, G., & Murawska-Ciałowicz, E. (2021). The role of satellite cells in skeletal muscle regeneration—the effect of exercise and age. Biology, 10(10), 1056. doi: https://doi.org/10.3390/biology10101056

Kamerkar, S. C., Liu, A., & Higgs, H. N. (2025). Mitochondrial fission–changing perspectives for future progress. Journal of cell science, 138(9), jcs263640. doi: https://doi.org/10.1242/jcs.263640

Kim, H.-J., So, B., Son, J. S., Song, H. S., Oh, S. L., Seong, J. K., . . . Song, W. (2015). Resistance training inhibits the elevation of skeletal muscle derived-BDNF level concomitant with improvement of muscle strength in zucker diabetic rat. Journal of exercise nutrition & biochemistry, 19(4), 281. doi: https://doi.org/10.5717/jenb.2015.15112402

Larsen, S., Nielsen, J., Hansen, C. N., Nielsen, L. B., Wibrand, F., Stride, N., . . . Dela, F. (2012). Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. The Journal of physiology, 590(14), 3349-3360. doi: https://doi.org/10.1113/jphysiol.2012.230185

Li, J., Wang, Z., Li, C., Song, Y., Wang, Y., Bo, H., & Zhang, Y. (2022). Impact of exercise and aging on mitochondrial homeostasis in skeletal muscle: roles of ROS and epigenetics. Cells, 11(13), 2086. doi: https://doi.org/10.3390/cells11132086

Mahatme, S., Vaishali, K., Kumar, N., Rao, V., Kovela, R. K., & Sinha, M. K. (2022). Impact of high-intensity interval training on cardio-metabolic health outcomes and mitochondrial function in older adults: a review. Medicine and Pharmacy Reports, 95(2), 115. doi: https://doi.org/10.15386/mpr-2201

Moore, T. M., Zhou, Z., Cohn, W., Norheim, F., Lin, A. J., Kalajian, N., . . . Ho, T. (2019). The impact of exercise on mitochondrial dynamics and the role of Drp1 in exercise performance and training adaptations in skeletal muscle. Molecular Metabolism, 21, 51-67. doi: https://doi.org/10.1016/j.molmet.2018.11.012

Musci, R. V., Fuqua, J. D., Peelor III, F. F., Nguyen, H. V. M., Richardson, A., Choi, S., . . . Wanagat, J. (2025). Age-induced changes in skeletal muscle mitochondrial DNA synthesis, quantity, and quality in genetically unique rats. Geroscience, 47(1),851-862.doi: https://doi.org/10.1007/s11357-024-01344-4

Phillips, S. M., Glover, E. I., & Rennie, M. J. (2009). Alterations of protein turnover underlying disuse atrophy in human skeletal muscle. Journal of applied physiology, 107(3), 645-654. doi: https://doi.org/10.1152/japplphysiol.00452.2009

Preminger, N., & Schuldiner, M. (2024). Beyond fission and fusion—Diving into the mysteries of mitochondrial shape. PLoS Biology, 22(7), e3002671. doi: https://doi.org/10.1371/journal.pbio.3002671

Qin, X., Li, H., Zhao, H., Fang, L., & Wang, X. (2024). Enhancing healthy aging with small molecules: A mitochondrial perspective. Medicinal research reviews, 44(4), 1904-1922. doi: https://doi.org/10.1002/med.22034

Radak, Z., Torma, F., Berkes, I., Goto, S., Mimura, T., Posa, A., . . . Higuchi, M. (2019). Exercise effects on physiological function during aging. Free Radical Biology and Medicine, 132, 33-41. doi: https://doi.org/10.1016/j.freeradbiomed.2018.10.444

Ringholm, S., Gudiksen, A., Frey Halling, J., Qoqaj, A., Meizner Rasmussen, P., Prats, C., . . . Pilegaard, H. (2023). Impact of aging and lifelong exercise training on mitochondrial function and network connectivity in human skeletal

muscle. The Journals of Gerontology: Series A, 78(3), 373-383. doi: https://doi.org/10.1093/gerona/glac164

Sebastián, D., Hernández-Alvarez, M. I., Segalés, J., Sorianello, E., Muñoz, J. P., Sala, D., . . . Gopalacharyulu, P. (2012). Mitofusin 2 (Mfn2) links mitochondrial and endoplasmic reticulum function with insulin signaling and is essential for normal glucose homeostasis. Proceedings of the National Academy of Sciences, 109(14), 5523-5528. doi: https://doi.org/10.1073/pnas.1108220109

Sheffield-Moore, M., Yeckel, C., Volpi, E., Wolf, S., Morio, B., Chinkes, D., . . . Wolfe, R. (2004). Postexercise protein metabolism in older and younger men following moderate-intensity aerobic exercise. American Journal of Physiology-Endocrinology And Metabolism, 287(3), E513-E522. doi: https://doi.org/10.1152/ajpendo.00334.2003

Sinha, J. K., Jorwal, K., Singh, K. K., Han, S. S., Bhaskar, R., & Ghosh, S. (2024). The potential of mitochondrial therapeutics in the treatment of oxidative stress and inflammation in aging. Molecular neurobiology, 1-16. doi: https://doi.org/10.1007/s12035-024-04474-0

Thomas, A. C., Brown, A., Hatt, A. A., Manta, K., Costa-Parke, A., Kamal, M., . . . Kumbhare, D. (2022). Short-term aerobic conditioning prior to resistance training augments muscle hypertrophy and satellite cell content in healthy young men and women. The FASEB Journal, 36(9), e22500. doi: https://doi.org/10.1096/fj.202200398RR

Wilkinson, S. B., Phillips, S. M., Atherton, P. J., Patel, R., Yarasheski, K. E., Tarnopolsky, M. A., & Rennie, M. J. (2008). Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. The Journal of physiology, 586(15), 3701-3717. doi: https://doi.org/10.1113/jphysiol.2008.153916

Yeo, R. X., Dijkstra, P. J., De Carvalho, F. G., Yi, F., Pino, M. F., Smith, S. R., & Sparks, L. M. (2022). Aerobic training increases mitochondrial respiratory capacity in human skeletal muscle stem cells from sedentary individuals. American Journal of Physiology-Cell Physiology. doi: https://doi.org/10.1152/ajpcell.00146.2022

Zhao, Y.-C., & Gao, B.-h. (2024). Integrative effects of resistance training and endurance training on mitochondrial remodeling in skeletal muscle. European journal of applied physiology, 124(10), 2851-2865. doi: https://doi.org/10.1007/s00421-024-05549-5

Zhong, Q., Zheng, K., Li, W., An, K., Liu, Y., Xiao, X., . . . An, Z. (2023). Post-translational regulation of muscle growth, muscle aging and sarcopenia. Journal of cachexia, sarcopenia and muscle, 14(3), 1212-1227. doi: https://doi.org/10.1002/jcsm.13241