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# The effects of different exercise training protocols on mitochondrial dynamics in skeletal and cardiac muscles of Wistar rats

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## Abstract

**Background** Mitochondrial fission and fusion both contribute to maintaining mitochondrial function and optimizing bioenergetic capacity.

**Objective** The aim of this study was to compare the effect of aerobic and resistance training on mitochondrial fission and fusion markers in skeletal and cardiac muscles of Wistar rats.

**Method** 24 male Wistar rats were randomly divided into four groups of moderate-intensity interval training (MIIT), high-intensity interval training (HIIT), resistance training (RT) and control (CON). The MIIT and HIIT groups performed treadmill exercises with an intensity of 60–65% and 80–85% of the maximum speed, respectively, while the RT group performed resistance training with an intensity of 30–60% of the rat's body weight for 8 weeks. The soleus (SOL), extensor digitorum longus (EDL) and left ventricular tissues were used to evaluate markers of mitochondrial fission and fusion PGC-1α (fusion/fission), Opa-1 (fusion), Fis-1 (fission), Drp-1 (fission), Mfn-1 and Mfn-2 (fusion) genes expression.

**Results** In all three tissues, a significant increase in some mitochondrial fusion markers was observed after 8 weeks of training (p = < 0.0001–0.0452). Furthermore, a significant decrease in cardiac mitochondrial fission markers was observed in all three groups (p = < 0.0001–0.0156). This reduction in some markers was evident in the SOL tissue of the HIIT group ($p < 0.0001$ for Drp-1 and $p = 0.0007$ for Fis-1) and in the EDL tissue of the RT group ($p = 0.0005$ for Fis-1 and $p = 0.0012$ for Drp-1). The mitochondrial fission/fusion markers in the heart ($p = 0.0007$–$0.0449$) and SOL ($p = 0.0050$–$0.0258$) tissues of the HIIT group had more changes than the RT group, while the mitochondrial fission markers in the EDL tissue of the RT group had a lower level than the HIIT ($p = 0.0087$ for Drp-1) and MIIT ($p = 0.0130$ for Fis-1 and $p = 0.0010$ for Drp-1) groups.

**Conclusion** Our study demonstrated that HIIT, through better regulation of mitochondrial fusion and fission than RT, improves mitochondrial dynamics in cardiac and SOL tissues.

**Keywords** Exercise, Mitochondrial dynamics, Muscles, Myocardium, Rats

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## Background

Mitochondria as highly plastic and dynamic organelles are constantly undergoing fission, mitophagy and transport cycles and fusion that determine the morphology, quality, quantity and distribution of mitochondria in cells as well as mitochondrial function [1]. Fission helps to create new mitochondria, facilitate apoptosis due to oxidative stress and increase the quality of this organelle by removing damaged mitochondria [2], while to maintain mitochondrial function and reduce stress, the fusion process helps to mix and exchange the intramitochondrial contents between mitochondria [1].

Each step of mitochondrial dynamics is regulated by upstream signaling cascades. Mitochondrial fusion is controlled by three GTPases such as mitofusin-1 (Mfn-1), mitofusin-2 (Mfn-2) and optic atrophy-1 (Opa-1). On the other hand, mitochondrial fission is regulated through dynamin-related protein-1 (Drp-1) and mitochondrial fission-1(Fis-1) [2]. Mfn-2, which plays a central role in fusion regulation [3], is activated through peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α), PGC-1β and Jun N-terminal kinase (JNK) [1]. Mfn-1 is activated by adenosine monophosphate-activated protein kinase (AMPK) and inactivated by reactive oxygen species (ROS) in the presence of Opa-1 as responsible for connecting adjacent mitochondria to each other [4]. Drp-1 is activated by increasing AMPK, calcineurin and extracellular-signal-regulated kinase 1/2 (ERK1/2) activity and inactivated by increasing mammalian target of rapamycin (mTOR), protein kinase A (PKA) and Sirtuin (SIRT) activity [1]. Drp-1 recruitment to mitochondria is probably facilitated by an array of specific adapter proteins located in the outer mitochondrial membrane, such as mitochondrial Fis-1 [5]. PGC-1α is a master regulator of mitochondrial biogenesis that provides a link between mitochondrial biogenesis and fission/fusion [5]. The transcriptional process is initiated when PGC-1α is activated by AMPK phosphorylation or SIRT1 deacetylation, which stimulates nuclear transcription factors such as nuclear respiratory factor 1 (NRF-1), NRF-2 and estrogen-related receptor alpha (ERRα) and induces the expression of mitochondrial transcription factor A (TFAM), then it is transferred to mitochondria to control mitochondrial DNA transcription and replication [6].

Muscle fibers in terms of contraction speed, myosin heavy chain (MHC) expression, and metabolic capacity differ from each other [7]. The extensor digitorum longus (EDL) muscle has a higher composition in MHC-IIb and IIx fibers compared to the soleus (SOL), which instead has a higher composition in MHC-I and IIa fibers [8]. Comparison of SOL and EDL muscles showed that mitochondrial content is greater in SOL muscle [9]. It seems that the protein content of MiD49 and Mfn-2 (as fission and fusion proteins, respectively) is higher in SOL muscle than EDL [9]. In rodent ventricular myocardium, MHC-α is the main isoform (~90%), which has higher actomyosin ATPase activity than MHC-β, which is the dominant isoform in human ventricular myocardium (~95%) [10].

It has been found that mitochondrial fission and fusion might be important for maintaining and possibly for increasing physical fitness with a bout of exercise [11]. Exercise by promoting mitochondrial adaptation leads to improvement of mitochondrial dynamics and clearance and promotion of biogenesis [1]. Exercise training adaptations depend on the intensity, length and type of exercise and type of muscle fiber [12, 13] and its effects on mitochondrial function are not well understood. Pengam et al. [13] who examined 6 weeks of moderate-intensity continuous training (MICT) versus high-intensity interval training (HIIT) showed that in SOL, Mfn-2, Opa-1 and Drp-1 mRNA levels in the HIIT group were at least 50% higher than in the untrained group. HIIT up-regulated Fis-1 transcription compared with MICT. No effects of training protocol were observed on these mitochondrial dynamics genes in EDL. In SOL, the training protocols did not change PGC-1α mRNA, but in EDL, PGC-1α mRNA content was significantly up-regulated by HIIT compared with untrained and MICT. Although resistance training (RT) is effective in increasing muscle strength and mass, as well as improving mitochondrial function and mitochondrial growth [14], its effect on mitochondrial dynamics is not well defined. RT for 10 weeks in vastus lateralis muscle did not change PGC-1α protein levels but increased the protein level of Mfn-1, Mfn-2, Opa-1 and Drp-1 [15]. In cardiac myocytes, mitochondria occupy approximately one-third of the cell volume, reflecting the high energy requirements of these cells [16]. The 8 weeks of aerobic interval treadmill running increased Mfn-1, Mfn-2, Opa-1 and on the other hand decreased Drp-1 in the cardiac tissue of rats [17]. Based on previous studies, the mitochondrial dynamics of oxidative muscles may change more than glycolytic ones as a result of exercise training [18]. It is also possible that HIIT causes a greater increase in AMPK gene expression than moderate-intensity interval training (MIIT) in oxidative muscles [19]. Therefore, which exercise protocol and/or which muscle exhibits the greatest change in mitochondrial dynamics is still unclear. The aim of this study was to compare the effect of aerobic training and RT on mitochondrial fission and fusion markers in skeletal and cardiac muscles of Wistar rats.

## Methods

### Ethics approval

This study was approved by the Ethics Committee of Hakim Sabzevari University, Iran with approval ID: IR.HSU.AEC.1401.017.

### Study design and animals

The present study is an experimental study with a control (CON) group. 24 male Wistar rats at the age of 8 weeks with an average weight of about 200 g were purchased from Pastor Institute, Babol, Iran and were randomly divided into CON, MIIT, HIIT and RT groups (6 rats in each group). The animals were kept under a sleep-wake cycle (12 h of light and 12 h of darkness) and at a temperature of 22 ± 3 °C and a humidity of 40 to 60%. Before the interventions, the rats were kept for one week to adapt to the environment. Food and water were provided freely to the rats. Rats of 4 groups were killed after the end of the intervention period.

### Exercise protocols

Training programs were designed for 8 weeks and 5 sessions per week (Table 1). Exercises included MIIT (60–65% of the maximum speed), HIIT (80–85% of the maximum speed) and RT (30–60% rat weight). The rats were familiarized with the exercise procedures 3 days a week before the intervention. Aerobic exercises were performed on a rodent treadmill (Pishro Andishe Sanat Co, model A1400Y10, Iran) with no inclination. RT included climbing a ladder (1 m, 2-cm grid, 85° incline) by carrying a weight in the rat's tail. Each training session in the MIIT and HIIT groups began with a six-min warm-up (20% of the maximum speed for MIIT and 40–60% of the maximum speed for HIIT) and ended with a six-min cool-down (20% of the maximum speed for MIIT and 30% of the maximum speed for HIIT). The CON group experienced walking on the treadmill for 15 min at a very low speed (< 5 m/min) to have the same conditions for environmental stress [20]. In order to evaluate the maximum speed, 5 min of warm-up (5 m/min) on the treadmill was conducted at the beginning, and the speed increased 5 m/min every 3 min, so that the animals were no longer able to run [21]. Then the average maximum speed was calculated to design the HIIT and MIIT programs. The state of exhaustion was determined through changes in behavioral indicators such as changes in the positions of rats relative to the treadmill, the reaction of rats to sound stimulation, electrical stimulation and slippage time [22].

### Necropsy and tissue Preparation

24 h after the last training session, the rats were anesthetized with intramuscular injection of ketamine 75 mg/kg and xylazine 10 mg/kg. The muscles of SOL, EDL and cardiac left ventricle were separated, frozen in liquid nitrogen and stored at -80 °C to evaluate genes expression.

### RNA extraction, cDNA synthesis and real-time PCR

50 mg of each tissue of SOL, EDL and cardiac left ventricle was placed in a microtube. Total cellular RNA was extracted using Trizol reagent (YTzol Pure RNA, Iran) according to the manufacturer's protocol. For this purpose, the samples were lysed with Buffer RB, homogenized with a homogenizer and washed with 75% ethanol. The quantity and purity of RNA were determined by measuring the absorbance at 260 nm and the A260/A280 ratio. The cDNA synthesis was prepared according to the instructions in the cDNA synthesis kit (Thermo Fisher

**Table 1** Characteristics of the training programs

| **High intensity interval training** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Weeks** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| Number of intervals | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Effort duration (min) | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Effort velocity (m/min) | 20 | 20 | 25 | 25 | 30 | 30 | 35 | 35 |
| Rest duration | 2 | 2 | 3 | 3 | 4 | 4 | 4 | 4 |
| Rest velocity (m/min) | 10 | 10 | 10 | 10 | 15 | 15 | 15 | 15 |
| **Moderate-intensity interval training** | | | | | | | | |
| **Weeks** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| Number of intervals | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Effort duration (min) | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Effort velocity (m/min) | 15 | 15 | 20 | 20 | 25 | 25 | 30 | 30 |
| Rest duration (min) | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Rest velocity (m/min) | 10 | 10 | 10 | 15 | 15 | 15 | 15 | 15 |
| **Resistance training** | | | | | | | | |
| **Weeks** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| Intensity (% rat weight) | 30 | 30 | 30 | 40 | 40 | 50 | 50 | 60 |
| Sets (n) | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Repeats (n) | 5 | 6 | 6 | 7 | 7 | 8 | 9 | 9 |
| Rest between sets (min) | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Rest between reps (min) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

**Table 2** Primer sequences

| Gene name | Forward primer | Primer size (bp) | Reverse primer | Primer size (bp) |
|---|---|---|---|---|
| Drp-1 | CGGCCTAGCTCAGGGTTTTA | 20 | TCTGCAGATTTGGGGCAAC | 19 |
| Fis-1 | CATCGTGCTGCTGGAGGA | 18 | TTGTTCTGGGGCTCAGTCTGT | 21 |
| Mfn-1 | CTAGAGGATGGCTGCACTAAACAC | 24 | AAGCAAACAGGGCCAATGTC | 20 |
| Mfn-2 | ATGTCTGTGTGTCACTTCC | 19 | CAATGACCCACTGTGAGATGA | 21 |
| Opa-1 | GGATCTGCTGTTGGAGGTGG | 20 | GTCTTCTGAACTGGGAAGGG | 20 |
| PGC-1α | CACCAAACCCACAGAGAACAG | 21 | GGTGACTCTGGGGTCAGAG | 19 |
| β-Actin | ACAAGCCACAGGATTACAAGAA | 22 | CACCAATGTCCAGTCCAAGA | 20 |

Mfn-1: mitofusin-1, Mfn-2: mitofusin-2, Opa-1: optic atrophy-1, PGC-1α: peroxisome proliferator-activated receptor gamma co-activator 1 alpha, Drp-1: dynamin-related protein-1, Fis-1: mitochondrial fission-1

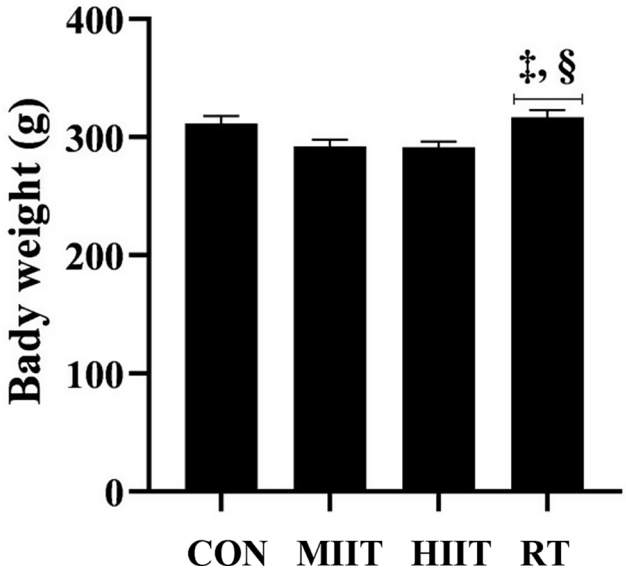


**Fig. 1** Final weight of rats in study groups (The data presented as mean ± SEM). ‡: significant differences between HIIT and RT groups, §: significant differences between MIIT and RT groups. CON: control, MIIT: moderate-intensity interval training, HIIT: high-intensity interval training, RT: resistance training

Scientific, Fermentas, USA, cat NO: K1621). The reverse transcription reaction was performed by RevertAid™ M-MuLV Reverse Transcriptase enzyme. Genes expression levels was measured by real-time PCR. RealQ 2x Master mix Green Dye (Ampliqon, Germany), cDNA and synthesized primers were used for this step. The amplification curve of the target gene was normalized with the amplification curve of the reference gene. The $2^{-\Delta\Delta CT}$ formula was also used to determine the genes expression. The sequence of primers used is shown in Table 2. β-Actin gene was also used as an internal reference gene. All of these primers were synthesized by Sinaclon Co., Iran.

### Statistical analysis

Data were tested for the normal distribution using Shapiro-Wilk test and for the homogeneityof variance by Brown-Forsythe test. When the variances were homogeneous, one-way ANOVA with Sidak's multiple comparisons test was used to compare between groups. When the variances were not homogeneous, Welch's ANOVA with Dunnett's T3 multiple comparisons test was used to compare between groups. All statistical analysis were also performed using GraphPad Prism software version 8 and a significant level of p-value < 0.05 was considered.

## Results

The final weight of the rats is shown in Fig. 1. The body weight after the training period in the RT group is more than the HIIT ($p = 0.0339$) and MIIT ($p = 0.0424$).

### The effect of training on the gene expression of mitochondrial indices in SOL muscle

The results showed that there was a significant difference between the CON, MIIT, HIIT and RT groups in Mfn-1($p = 0.0004$), Mfn-2 ($p < 0.0001$), Opa-1 ($p = 0.0011$), PGC-1α ($p = 0.0002$), Drp-1 ($p = 0.0002$) and Fis-1 ($p = 0.0002$) genes expression.

In the HIIT group, PGC-1α ($p = 0.0037$), Opa-1 ($p = 0.0010$), Mfn-1 ($p = 0.0206$) and Mfn-2 ($p = 0.0011$) genes expression significantly increased compared to CON, while Fis-1 ($p = 0.0007$) and Drp-1 ($p < 0.0001$) genes expression decreased. PGC-1α ($p = 0.0281$), Mfn-1($p = 0.0452$) and Mfn-2 ($p = 0.0075$) genes expression significantly increased in the MIIT group compared to CON. In the RT group, Opa-1 ($p = 0.0149$), Mfn-1($p = 0.0158$) and Mfn-2 ($p = 0.0020$) genes expression increased compared to CON. PGC-1α ($p = 0.0133$) and Mfn-2 ($p = 0.0050$) genes expression significantly increased in the HIIT group compared to RT, and on the contrary, Fis-1 ($p = 0.0258$) and Drp-1 ($p = 0.0129$) genes expression had a significant decrease. Drp-1 gene expression was lower in HIIT group than MIIT ($p = 0.0367$; Fig. 2).

### The effect of training on the gene expression of mitochondrial indices in EDL muscle

The results showed that there was a significant difference between the CON, MIIT, HIIT and RT groups in Mfn-1($p = 0.0006$), Mfn-2 ($p < 0.0001$), Opa-1 ($p = 0.0022$),

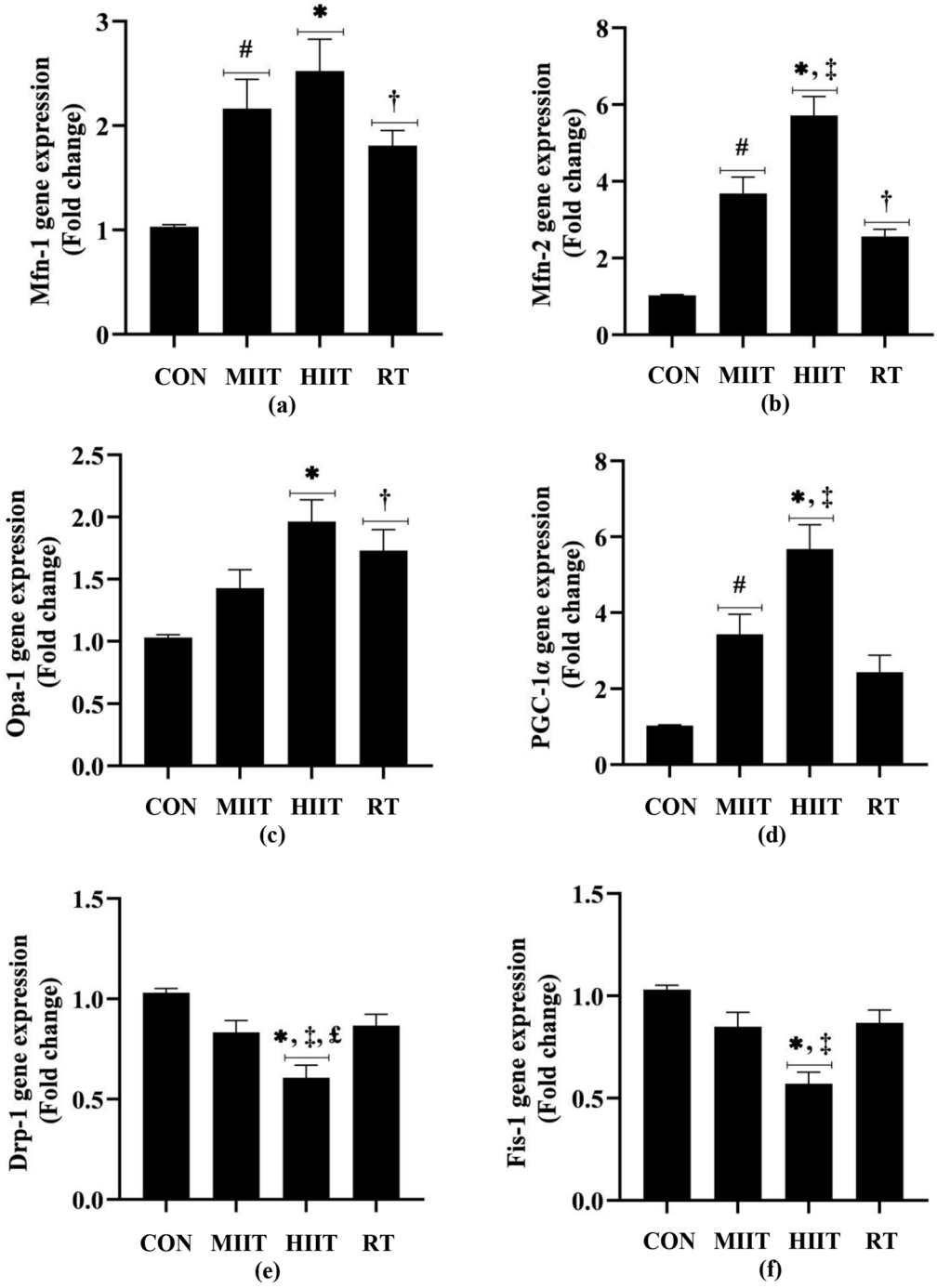


**Fig. 2** The effect of training on the gene expression of mitochondrial indices in SOL muscle (The data presented as mean ± SEM). (**a**) Mfn-1 gene expression, (**b**) Mfn-2 gene expression, (**c**) Opa-1 gene expression, (**d**) PGC-1α gene expression, (**e**) Drp-1 gene expression, (**f**) Fis-1 gene expression. *significant differences between HIIT and CON groups, #: significant differences between MIIT and CON groups, †: significant differences between RT and CON groups, ‡: significant differences between HIIT and RT groups, £: significant differences between HIIT and MIIT groups. CON: control, MIIT: moderate-intensity interval training, HIIT: high-intensity interval training, RT: resistance training, SOL: soleus, Mfn-1: mitofusin-1, Mfn-2: mitofusin-2, Opa-1: optic atrophy-1, PGC-1α: peroxisome proliferator-activated receptor gamma co-activator 1 alpha, Drp-1: dynamin-related protein-1, Fis-1: mitochondrial fission-1

PGC-1α ($p < 0.0001$), Drp-1 ($p = 0.0002$) and Fis-1 ($p = 0.0007$) genes expression.

In the HIIT group, PGC-1α ($p = 0.0289$), Opa-1 ($p = 0.0039$), Mfn-1 ($p = 0.0006$), Mfn-2 ($p = 0.0245$) genes expression increased compared to CON. PGC-1α ($p < 0.0001$) and Mfn-2 ($p = 0.0058$) genes expression increased in the MIIT group compared to CON. In the RT group, PGC-1α ($p = 0.0099$), Opa-1 ($p = 0.0067$),

Mfn-1 ($p = 0.0055$) and Mfn-2 ($p = 0.0050$) genes expression increased compared to CON, while, Fis-1 ($p = 0.0005$) and Drp-1 ($p = 0.0012$) genes expression decreased. Drp-1 gene expression was significantly decreased in RT group compared to HIIT ($p = 0.0087$) and MIIT ($p = 0.0010$). Fis-1 gene expression in RT group was lower than MIIT ($p = 0.0130$; Fig. 3).

### The effect of training on the gene expression of mitochondrial indices in heart muscle

The results showed that there was a significant difference between the CON, MIIT, HIIT and RT groups in Mfn-1 ($p < 0.0001$), Mfn-2 ($p < 0.0001$), Opa-1 ($p = 0.0003$), PGC-1α ($p < 0.0001$), Drp-1 ($p < 0.0001$) and Fis-1 ($p = 0.0003$) genes expression.

In the HIIT group, PGC-1α ($p < 0.0001$), Opa-1 ($p = 0.0083$), Mfn-1 ($p < 0.0001$) and Mfn-2 ($p = 0.0019$) genes expression increased compared to CON, while, Fis-1 ($p = 0.0002$) and Drp-1 ($p < 0.0001$) genes expression decreased. PGC-1α ($p < 0.0001$) and Mfn-1 ($p = 0.0098$) genes expression in the MIIT group increased significantly compared to CON, and on the contrary, Fis-1 ($p = 0.0152$) and Drp-1 ($p = 0.0084$) genes expression decreased. In the RT group, PGC-1α ($p = 0.0010$), Opa-1 ($p = 0.0114$) and Mfn-2 ($p = 0.0106$) genes expression increased significantly compared to CON, and on the contrary, Drp-1 gene expression had a significant decrease ($p = 0.0156$). In the HIIT group, PGC-1α ($p = 0.0002$), Opa-1 ($p = 0.0449$), Mfn-1 ($p = 0.0069$) and Mfn-2 ($p = 0.0148$) genes expression increased significantly compared to RT, and on the contrary, Fis-1 ($p = 0.0351$) and Drp-1 ($p = 0.0007$) genes expression had a significant decrease (Fig. 4).

## Discussion

The aim of this study was to compare the effect of MIIT, HIIT and RT on mitochondrial fission and fusion markers in skeletal and cardiac muscles of Wistar rats. In the SOL muscle, most of the mitochondrial fusion markers increased in different training programs, but the mitochondrial fission markers decreased only in HIIT. Most of the mitochondrial fusion markers were increased in HIIT compared to RT. Also, most of mitochondrial fission markers in HIIT had a greater decrease than in RT and MIIT. In the EDL muscle, most of the mitochondrial fusion markers increased in different training programs, but the mitochondrial fission markers decreased only in RT. Some mitochondrial fission markers in RT had a greater decrease than in HIIT and MIIT. In the heart muscle, most of the mitochondrial fission/fusion markers improved in different training programs. All markers of mitochondrial fission/fusion improved in HIIT compared to RT.

The findings are consistent with the fact that predominantly slow-twitch (oxidative) muscles have larger and more numerous mitochondria, slower contraction rates, greater reliance on oxidative phosphorylation, and higher resistance to fatigue than predominantly fast-twitch (glycolytic) muscles [18]. Mitochondrial dynamics depend on the fiber type distribution in the muscle, so that it has been determined that mitochondrial fusion rates are related to oxidative capacity at the fiber level. In this context, oxidative-dependent fibers contain elongated mitochondrial networks with higher fusion rates [18]. This content is in line with the results of our study because the expression of mitochondrial fusion proteins Mfn-2 and Opa-1 in HIIT was higher in slow-twitch than fast-twitch muscles. It has been found that chronic muscle use increases the fusion/fission proteins ratio, resulting in reticular mitochondria, while muscle disuse leads to a decrease in this ratio, culminating in fragmented organelles [23]. In agreement with this theory, the results of our study showed that the expression of fusion proteins increased during different training protocols, which is more than untrained rats.

Moore et al. [24] showed that 30 days of in-cage voluntary wheel running does not cause significant changes in Opa-1, Fis-1, Drp-1, Mfn-1 and Mfn-2 expressions. A significant decrease in Drp-1 protein was observed in diabetic cardiomyopathy mice after 5 weeks of moderate intensity exercise, while Mfn-2 protein was unchanged [25]. Mesquita et al. [15] investigated the effect of 10 weeks of RT with three sets of 10–12 repetitions on the changes in the indices of mitochondrial biogenesis, fusion and fission of the vastus lateralis muscle in the untrained elderly. They concluded that PGC-1α protein levels do not change due to exercise, but the protein levels of Mfn-1, Mfn-2, Opa-1 and Drp-1 increase. A significant increase in total PGC-1α gene expression was observed in the SOL muscle of healthy mice after 6 weeks of endurance training [26], which was in agreement with the results of the present study. In fact, D'Amico et al. [8] demonstrated in mice that in the soleus, which is a muscle rich in type IIa fibers, resistance exercise specifically activates genes involved in mitochondrial biogenesis such as the PGC1 α1 isoform, Hsp60 and IL-6, while the expression of PGC1 α2 and α3 was significantly increased in the EDL muscle, a fast-twitch skeletal muscle. Furthermore, in the study of Pengam et al. [13] showed that mitochondrial fusion markers increased in SOL as a result of HIIT, which was in agreement with the results of the present study. On the other hand, Drp-1 also had a significant increase, which was contrary to our results. These discrepancies may be related to the mobility of the rats in the cage before the intervention and the intensity and duration of the training program. In the study of Pengam et al. [13] HIIT intensity was between 85

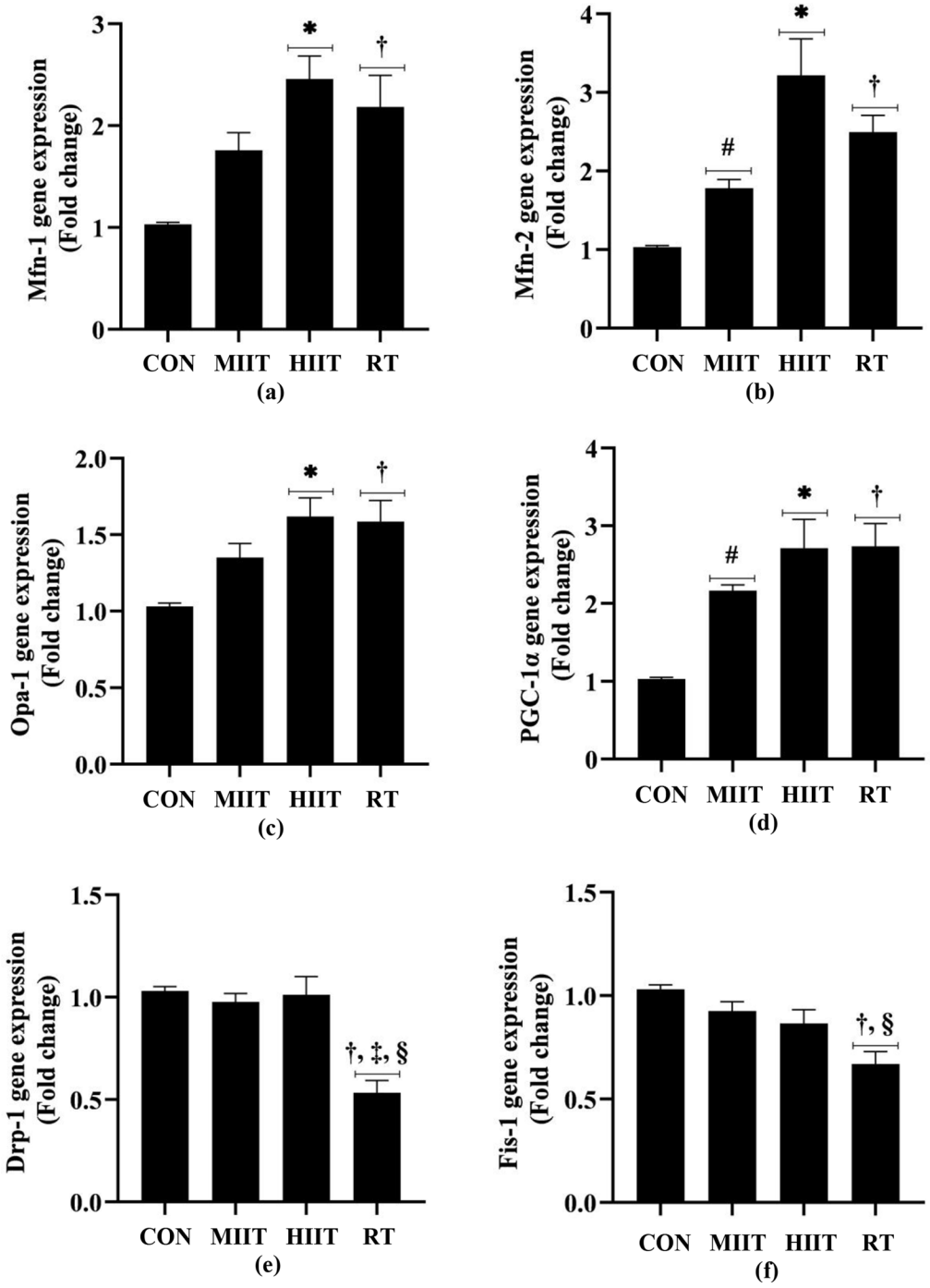


**Fig. 3** The effect of training on the gene expression of mitochondrial indices in EDL muscle (The data presented as mean ± SEM). (**a**) Mfn-1 gene expression, (**b**) Mfn-2 gene expression, (**c**) Opa-1 gene expression, (**d**) PGC-1α gene expression, (**e**) Drp-1 gene expression, (**f**) Fis-1 gene expression. *significant differences between HIIT and CON groups, #: significant differences between MIIT and CON groups, †: significant differences between RT and CON groups, ‡: significant differences between HIIT and RT groups, §: significant differences between MIIT and RT groups. CON: control, MIIT: moderate-intensity interval training, HIIT: high-intensity interval training, RT: resistance training, EDL: extensor digitorum longus, Mfn-1: mitofusin-1, Mfn-2: mitofusin-2, Opa-1: optic atrophy-1, PGC-1α: peroxisome proliferator-activated receptor gamma co-activator 1 alpha, Drp-1: dynamin-related protein-1, Fis-1: mitochondrial fission-1

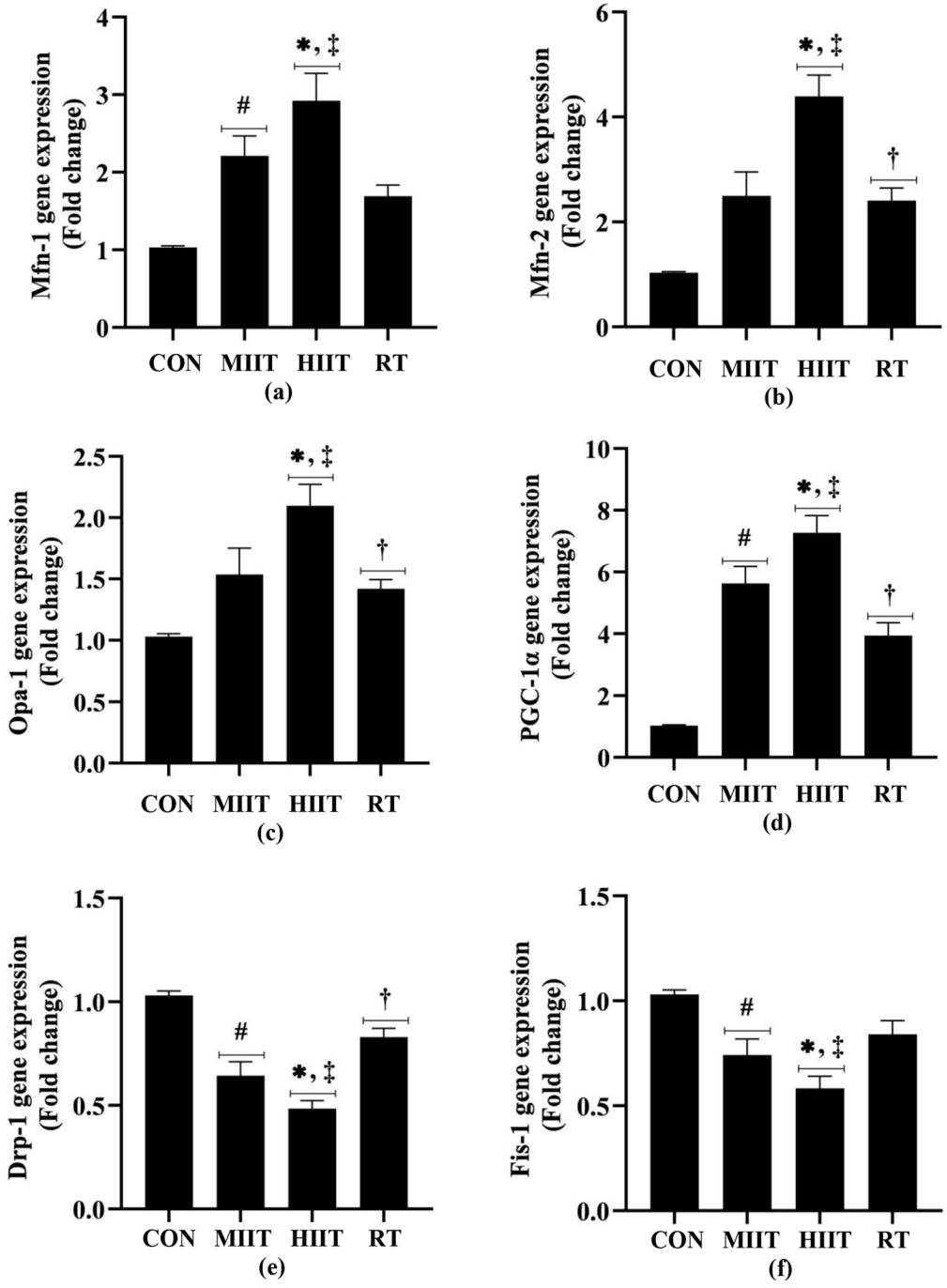


**Fig. 4** The effect of training on the gene expression of mitochondrial indices in heart muscle (The data presented as mean ± SEM). (**a**) Mfn-1 gene expression, (**b**) Mfn-2 gene expression, (**c**) Opa-1 gene expression, (**d**) PGC-1α gene expression, (**e**) Drp-1 gene expression, (**f**) Fis-1 gene expression. *significant differences between HIIT and CON groups, #: significant differences between MIIT and CON groups, †: significant differences between RT and CON groups, ‡: significant differences between HIIT and RT groups. CON: control, MIIT: moderate-intensity interval training, HIIT: high-intensity interval training, RT: resistance training, Mfn-1: mitofusin-1, Mfn-2: mitofusin-2, Opa-1: optic atrophy-1, PGC-1α: peroxisome proliferator-activated receptor gamma co-activator 1 alpha, Drp-1: dynamin-related protein-1, Fis-1: mitochondrial fission-1

and 90% of the maximum speed and the training duration was 6 weeks, while in our study the training intensity was between 80 and 85% of the maximum speed and the training duration was 8 weeks. Flockhart et al. [27] showed that excessive exercise causes mitochondrial dysfunction in healthy subjects. Despite the increase in mitochondrial fusion markers, the Fis-1 marker also increased, while the Drp-1 marker remained unchanged. This indicates that the intensity of HIIT in our study was not high enough to increase mitochondrial fission.

Increasing cellular energy demand is one of the factors that enhance mobility and fusion of mitochondria [28]. Although we did not measure it, AMPK is activated in response to energy stress by increasing AMP: ATP and ADP: ATP ratios and restores energy balance by inhibiting anabolic processes [29]. Both aerobic training and RT increase the AMPK protein expression, but aerobic exercises increase this protein more than RT [30]. This is probably the reason for the higher increase of mitochondrial fission markers in the heart and SOL tissue of the HIIT group compared to RT. In addition, AMPK can activate PGC-1α by increasing nicotinamide phosphoribosyltransferase (NAMPT) or through upregulation of mitochondrial fatty acid β-oxidation (FAO) or p38 mitogen-activated protein kinase (p38 MAPK) [31]. In addition to AMPK, many proteins affect the expression of PGC-1α, including cAMP-response element binding protein (CREB) and transducer of regulated CREB-binding protein (TORC), SIRT1 and general control non-depressible 5 (GCN5), protein kinase B (PKB), glycogen synthase kinase-3 beta (GSK-3β) [32]. Upregulation of PGC-1α simultaneously increases the expression of Mfn-2 and Opa-1 while inhibiting the expression of Fis-1 and Drp-1, thereby maintaining the balance between mitochondrial fission and fusion [32]. Other factors may have affected mitochondrial dynamics that we did not measure and should be considered as research limitations, possibly including ROS, JNK, ERK, mTOR, PKA, and SIRT. Lack of evaluation of proteins by western blot method was one of the main limitations of this study.

## Conclusions

Eight weeks of MIIT, HIIT and RT in rats has increased most markers of mitochondrial fusion in skeletal and cardiac muscle tissues and decreased some markers of mitochondrial fission. However, it seems that in order to increase the fusion and reduce the mitochondrial fission in slow-twitch skeletal and cardiac muscles, HIIT is preferable compared to RT. Further studies will be needed to confirm these data and investigate an improvement in mitochondrial dynamics also in human subjects.

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**Author contributions**
Conceptualization, A.H.H. and R.A.; Methodology, A.H.H. and M.R.B.; Validation, R.B., P.F., R.B. and M.A.P.; Visualization, R.B., P.F., R.B. and M.A.P.; Writing– review & editing, A.H.H., H.S. and M.A.P.

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**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethical approval**
The animal study protocol was approved by the Institutional Review Board of Hakim Sabzevari University (protocol code IR.HSU.AEC.1401.017 on January 21th, 2023).

**Consent for publication**
Not applicable in this section.

**Competing interests**
The authors declare no competing interests.

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