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Beneficial Mitochondrial Biogenesis in Gastrocnemius Muscle Promoted by High-Intensity Interval Training in Elderly Female Rats

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Abstract

Objective: Exercise can attenuate mitochondrial dysfunction caused by aging. Our study aimed to compare 12 weeks of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) on the expression of mitochondria proteins [e.g., AMP-activated protein kinase (AMPK), Estrogen-related receptor alpha (ERRα), p38 mitogen-activated protein kinase (P38MAPK), and Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α)] in gastrocnemius muscle of old female rats.

Materials and Methods: In this experimental study, thirty six old female Wistar rats (18-month-old and 270-310 g) were divided into three groups: i. HIIT, ii. MICT, and iii. Control group (C). The HIIT protocol was performed for 12 weeks with 16-28 minutes (2 minutes training with 85-90% VO_{2max} in high intensity and 2 minutes training with 45-75% VO_{2max} low intensity). The MICT was performed for 30-60 minutes with the intensity of 65-70% VO_{2max}. The gastrocnemius muscle expression of AMPK, ERRα, P38MAPK, and PGC1α proteins were determined by Western blotting.

Results: The expression of AMPK (P=0.004), P38MAPK (P=0.003), PGC-1α (P=0.028), and ERRα (P=0.006) in HIIT was higher than C group. AMPK (P=0.03), P38MAPK (P=0.032), PGC-1α (P=0.015), and ERRα (P=0.028) in MICT was higher than the C group. Also expression of AMPK (P=0.008), P38MAPK (P=0.009), PGC-1α (P=0.020) and ERRα (P=0.014) in MICT was higher than MICT group.

Conclusion: It seems that exercise training has beneficial effects on mitochondrial biogenesis, but the HIIT training method is more effective than MICT in improving mitochondrial function in aging.

Keywords: Aging, Exercise, Mitochondrial, Muscle

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Introduction

Aging is a natural, progressive and damaging process that is associated with various damages and reduction of functional efficiency in cells and tissues homeostasis over time (1, 2). Any discovery in cellular and molecular biology introduces a new family of aging theories, all of which will increase our perception of the aging process (3). Studies have shown that sarcopenia, or muscle atrophy, is associated with impaired muscle cell metabolism and disorder in ATP/ADP ratio, activated kinase protein of AMP (AMPK); This process is mediated by increased tumor necrosis factor alpha (TNF-α), oxidative stress and anabolic signaling pathways, such as Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α), p38 mitogen-activated protein kinases (P38MAPK)/Extracellular signal-regulated kinase (ERK)1/2 and c-Jun N-terminal kinases (JNKs) (4, 5). Almost all aging theories enumerate mitochondria dysfunction as one of the most critical factors in the aging process. This mitochondrial dysfunction is a multifactorial process in which DNA damage and enzymatic disorder are the most important processes (6). AMPK and P38MAPK are vital in Mitochondrial biogenesis. One of its downstream proteins, PGC1-α, is a multitask protein serving as a switch molecule, and this protein can increases the biogenesis of mitochondria by activating transcription factors such as nuclear respiratory factors 1 and 2 (NRF-1/2) and estrogen-related receptor alpha (ERRα) (7). Activation of these replicating factors increases the expression of mitochondrial transcription factor A (Tfam) and Mitochondrial transcription factor B1 and B2 (TFB1/2M). NRF1-2 is necessary to duplicate, replicate and maintain mtDNA (8, 9). Aging decrease the content of mitochondrial proteins (10). The researchers stated that the disorder of mitochondrial function in aging rats with telomere disorder had been correlated with rates of AMPK and PGC-1α suppression (6).

But regular exercise/training is a critical therapeutic intervention for aging, and it can improve brain function and metabolic disorders, attenuate sarcopenia, and increase mitochondrial oxidative capacity (11). According to studies, exercise training can lead to insulin-like growth factor-1 (IGF-1)/Ak/mTOR signaling activation mechanisms, as well as FoxOs/Nuclear transcription factor kappa-B (NF-κB) and MAPKs. Also, exercise

training increases NRF1/2, improves protein synthesis, and increases mitochondrial biogenesis in skeletal muscle (5, 12). The net effect of exercise depends on its variables; among them, intensity is the most important. Intense exercise training can lead to a higher reduction in ADP & AMP levels, making a better stimulation for AMPK activation.

Moreover, reports indicate that the type, intensity, and duration of exercise affect the expression of mitochondrial biogenesis proteins (8). Short-term, high-intensity endurance exercise and long-term aerobic swimming training increased SIRT1 and PGC-1α proteins in muscles (9). Another study showed the HIIT, and MICT drastically increased protein contents of PGC-1α and mtDNA expression. Soleus muscle was decreased in both inactive elderly rats versus inactive juveniles (10). Soleus muscle protein PGC-1α increased 2.3-fold in active elderly mice compared to inactive elderly mice (11, 13, 14). It has also been reported that the expression of mitochondrial biogenesis proteins, especially MAPK, NRF1-2, Tfam, SIRT1, and PGC1α, increased significantly in young people following high-intensity interval training (HIIT) compared to moderate intensity continuous training (MICT) (9). Although studies have examined the effect and intensity of endurance training on mitochondrial biogenesis in skeletal muscle, it seems that the difference in the type and intensity of exercise and its effect on mitochondrial function markers in sarcopenia is not well understood; in addition, due to the differences during the training period and adaptation to training, conducting studies with long-term training is very important in obtaining more information compared to the two types of exercise. So this study aimed to determine the effects of 12 weeks of HIIT and MICT on the expression of mitochondria proteins in old female rats.

Materials and Methods

This experimental study was performed in compliance with all ethical principles of working with laboratory animals and was approved by the Ethics Committee of the Faculty of Physical Education, University of Tehran (IR.UT.SPORT.REC.1397.021). In this study, 36 female elderly Wistar rats (18-month-old) (1, 15) were bought from the Pasteur Institute of Iran and randomly divided into three groups: HIIT, MICT, and control (C) groups. Rodent housed in a standard lab (12-hour light-dark cycle with an average temperature of 22-23°C and a humidity of 45%) with free access to water and food in the Faculty of Physical Education, University of Tehran. In addition, the rats in this study were kept in washable polycarbonate cages; a sterile wood grater was used to absorb moisture from the cages. All protocols conformed with the Guide Laboratory Animals for The Care and Use.

Maximum oxygen consumption determination

Rats were introduced to living conditions in the laboratory and trained on how to run on a treadmill for five days. The rat's maximum oxygen consumption (VO_{2max}) was assessed using an incremental test described by Høydal et al. (16). In summary, rats warmed up for 10 minutes at a speed of 10 meters per minute. Subsequently, they entered the test stage. Every two minutes, the treadmill's speed increased by 0.03 m/s until the rats could not continue the test (unable to run on the treadmill and go to the end space of the treadmill). After the exercise test was completed, the velocity at which the rat ran (last velocity in the exhaustion phase) was recorded. The VO_{2max} was measured using the equation y=162x-1 where y represents oxygen consumption in milliliters per minute per kilogram of body weight and x, indicates the maximum speed of running per meters per second).

Exercise protocol

Both HIIT and MICT protocols consisted of three parts: warm-up, main body, and cool down, as described in Tables 1 and 2. The following formulas were used to equalize the training load. Total training time in HIIT group: (minutes*intensity of work interval)+(minutes*intensity of rest interval). Total training time in MICT group: minutes*intensity).

Table 1: High-intensity interval training (HIIT) protocol

Week	Warm up	Work interval	Rest interval	Cool down	Total training time
1st and 2nd	3 minutes with running 45-50% VO_{2max}	4* (two-minute running with 85-90% VO_{2max}	4 two-minutes repetitions 45-50% VO_{2max}	3 minutes with running 45-50% VO_{2max}	14 minutes
3rd, 4th, and 5th	3 minutes- 45-50% $VO_{2\,max}$	5 two-minute repeated training 85-90% $VO_{2\,max}$	5 two-minutes repetitions 45-50% $VO_{2\,max}$	3 minutes with 45-50% intensity $VO_{2\,max}$	16 minutes
6th, 7th, and 8th	3 minutes- 45-50% VO_{2max}	7 two-minute repeated training 85-90% $VO_{2\,max}$	7 two-minutes repetitions 45-50% $VO_{2\,max}$	3 minutes with 45-50% intensity $VO_{2\,max}$	20 minutes
9th, 10th, 11th, and 12th	3 minutes- 45-50% VO_{2max}	7 two-minute repeated training 85-90% $VO_{2\,max}$	7 two-minutes repetitions 45-50% $VO_{2\,max}$	3 minutes with 45-50% intensity $VO_{2\,max}$	20 minutes

Table 2: MICT protocol

Training weeks	Warm up	Training body	Cool down	Total training time
Two first weeks	3 minutes, 45-50%VO_{2max}	65-70% VO_{2max} for 30 minutes	3 minutes, 45-50% VO_{2max}	36 minutes
3rd, 4th and 5th weeks	3 minutes, 45-50%VO_{2max}	65-70% VO_{2max} for 45 minutes	3 minutes, 45-50% VO_{2max}	51 minutes
6th, 7th, 8th weeks	3 minutes, 45-50%VO_{2max}	65-70% VO_{2max} for 50 minutes	3 minutes, 45-50% VO_{2max}	56 minutes
9th, 10th, 11th and 12th weeks	3 minutes, 45-50%VO_{2max}	65-70% VO_{2max} for 60 minutes	3 minutes, 45-50% VO_{2max}	66 minutes

Measurement procedures

Two days after the last training session, rats were anesthetized using ketamine and xylazine. Their left gastrocnemius muscle was extracted and immediately frozen in liquid nitrogen and kept at -80˚C until further analysis. Western blot was used to study AMPK, ERRα, P38MAPK, and PGC1α proteins levels. First, 100 mg of gastrocnemius muscle tissue was homogenized with 200 µl of Rippa lysis buffer, and then, the sample was centrifuged for 20 minutes at 1300 rpm at 4˚C. The supernatant was transferred to a 1 cc microtype, and 0.5 l was poured into another microtype to determine the protein concentration using nanodrop. The protein concentration in all samples was diluted with a certain ratio. Stacking gel and separating gel required for agarose gels were prepared using the materials needed. Subsequently, wells were created to pour the sample. Samples were poured into wells and placed in X1 electrophoresis buffer. After this step, the protein was transferred from the gel to PVDF paper. The primary antibodies AMPK (Ab191838, Cambridge Science Park, Cambridge, UK) and ERRα (Ab131607, Cambridge Science Park, Cambridge, UK) were diluted 1 to 1000 and used. After 24 hours of PVFD paper quenching, a diluted secondary antibody of 1 to 1000 was used. GAPDH was also used as a control protein, and finally, colored substrate (ECL) was added to PVDF paper in the dark room. After a few seconds, the emergence films were placed on paper. The emergence films were immediately transferred to the fixation and emergence solutions. After the desired protein bands appeared on the film strips, the films were photographed, and the size of each band was calculated using Image J software.

Statistical analysis

Shapiro-Wilk test was used to assess the data normality, and one-way analysis of variance (ANOVA) and Tukey post hoc tests were used to compare the data between groups. Data analysis was performed using SPSS software version 16 packaged by National Opinion Research Center (NORC), at the University of Chicago, and Microsoft Office software such as Excel and PowerPoint was used to design the graphs. The significance level was considered less than 0.05.

Results

The results of ANOVA showed that there was a significant difference between groups in AMPK (P=0.001), ERRα (P=0.001), P38MAPK (P=0.0013), and PGC1α (P=0.002).

Tukey's post hoc test showed that AMPK in both HIIT (P=0.004) and MICT (P=0.03) groups was higher than the C group and in HIIT was higher than the MICT group (P=0.008, Fig.1).

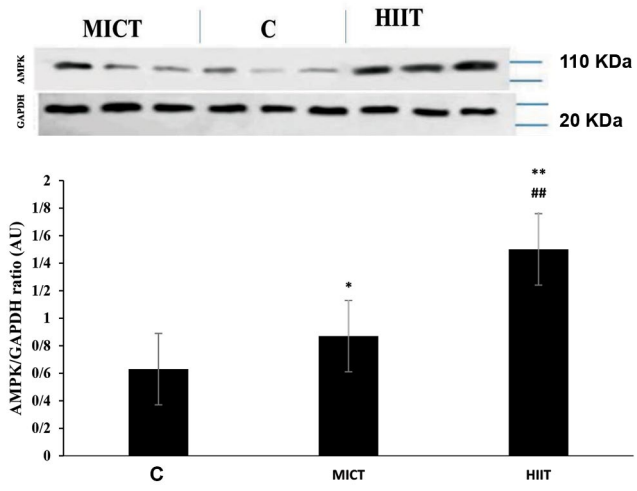

Fig.1: The ANOVA test results for expression rates of AMPK/GAPDH in research groups. *; P≤0.05, **; P≤0.01 increased AMPK in MICT and HIIT groups compared to the C group, and ##; P≤0.01 increased AMPK in the HIIT group compared to the MICT group. MICT; Moderate-intensity continuous training and HIIT; High-intensity interval training.

Also, the levels of P38MAPK protein in the MICT (P=0.032) and HIIT (P=0.003) groups significantly increased compared to the C group. Also, the levels of P38MAPK protein in the HIIT group showed a significant

increase compared to the MICT group (P=0.009, Fig.2).

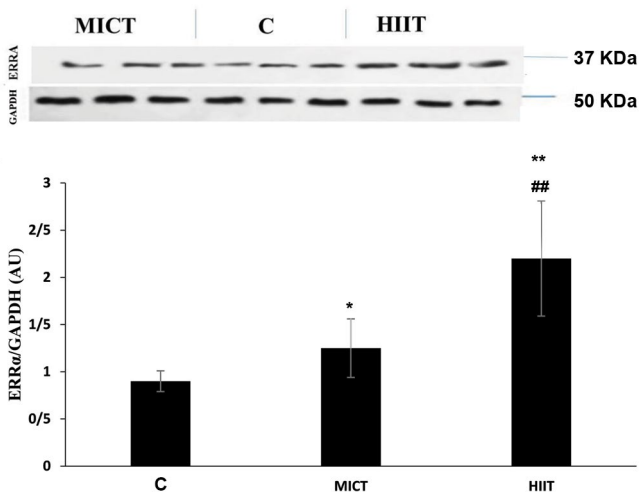

Fig.2: The ANOVA test results for expression rates of ERRa/GAPDH in research groups. *; P≤0.05, **; P≤0.01 increased ERRa in MICT and HIIT groups compared to the C group, and ##; P≤0.01 increased ERRa in the HIIT group compared to the MICT group. MICT; Moderate-intensity continuous training and HIIT; High-intensity interval training.

The level of PGC-1α protein in the MICT (P=0.015) and HIIT (P=0.028) groups significantly increased compared to the C group. The level of PGC-1α protein in the HIIT was higher than the MICT group (P=0.020, Fig.3).

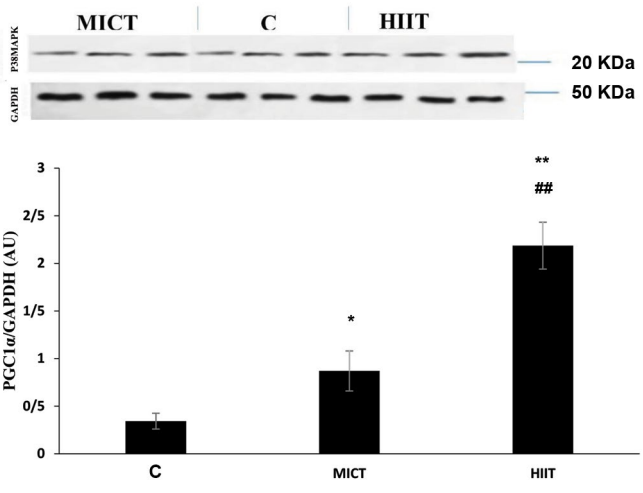

Fig.3: The ANOVA test results for expression rates of PGC1-a/GAPDH in research groups. *; P≤0.05, **; P≤0.01 increased PGC1-a in MICT and HIIT groups in comparison to the C group, and ##; P≤0.01 increased PGC1-a in the HIIT group compared to the MICT group.

Also, ERRα protein in the MICT (P=0.028) and HIIT (P=0.006) groups was higher than in the C group. ERRα protein levels in the HIIT group increased significantly compared with the MICT (P=0.014) group (Fig.4).

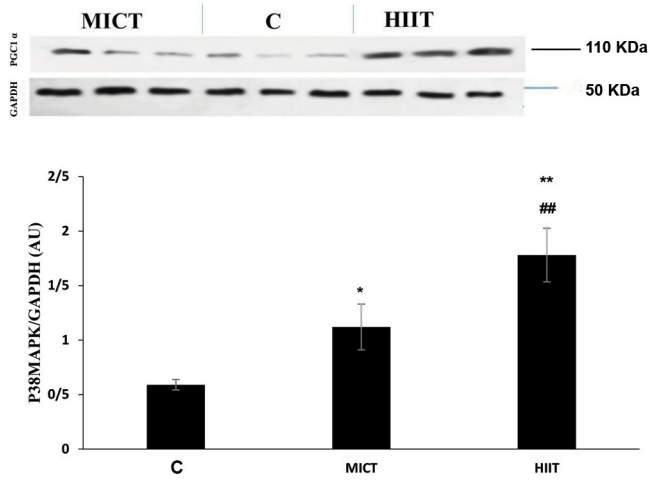

Fig.4: ANOVA test results for expression rates of P38MAPK/GAPDH in research groups. *; P≤0.05, **; P≤0.01 increased P38MAPK in MICT and HIIT groups compared to the C group, and ##; P≤0.01 increased P38MAPK in the HIIT group compared to the MICT group. MICT; Moderate-intensity continuous training and HIIT; High-intensity interval training.

Discussion

In this study, the effect of HIIT and MICT on total protein levels of AMPK, ERRα, P38MAPK, and PGC1α in the gastrocnemius muscle of aging rats was compared. This study showed that both HIIT and MICT models increased the amounts of proteins involved in mitochondrial biogenesis. The population of elderly women in the last century has increased due to increased life expectancy. With the onset of menopause in these women, estrogen secretion is disrupted; this factor is one of the leading causes of problems in elderly women, such as osteoporosis, sarcopenia, muscle atrophy, and decreased physical function (4). Physical activity increases oxidative capacity and muscle function. This adaptation is partly due to the exercise-induced increase in proteins involved in the transport and oxidation of metabolic substrates and the increase in mitochondrial content. Therefore, exercise may improve cellular metabolism. The mechanism of mitochondrial biogenesis seems to be multifactorial. Still, exercise intensity is one of the most critical factors in activating proteins in this pathway. The important finding of this study was that HIIT increased the amounts of AMPK, ERRα, P38MAPK, and PGC1α in the gastrocnemius muscle of aging rats more than MICT. These results highlight the importance of intensity in exercise-induced mitochondrial biogenesis. In line with our results, it has been shown that high-intensity exercise causes more cellular stress than low-intensity exercise, providing better stimulation for mitochondrial biogenesis (17). Granata et al. (18) showed that sprint interval training (SIT) had a more significant effect on mitochondrial function than HIIT.

Improving the lactate threshold could lead to better mitochondrial function, and because SIT is more effective in enhancing the lactate threshold, it causes more improvements in mitochondrial function. In other words, increasing exercise intensity enhances ADP and AMP production, which in turn increases ADP, AMP/ATP ratio and activates AMPK, an upstream of ERRα and PGC-1a (10). In addition to AMPK, it is also possible that activation of beta (2) -adrenergic receptor by catecholamines stimulates PGC-1α expression through an increase in cAMP levels and thus activation of CREB (AMP-responsive element-binding) transcription factors (19, 20). It seems that the same happens in aging rates because three weeks of endurance training with 85-90% of VO_{2max} increased the expression of PGC-1α, Tfam, and AMPK in the Soleus muscle (21, 22). HIIT training compared to MICT leads to a more significant increase in aerobic capacity (21). Increased cardiorespiratory capacity seems to be associated with increased stroke volume and cardiac output (23).

Furthermore, increased stroke volume and cardiac output are likely associated with the more significant cardiac challenge due to HIIT exercise program rotations. Increasing VO_{2max} leads to a further increase of oxidative capacity and, ultimately, more mitochondrial content. Therefore, one of the reasons for the further increase in PGC-1α protein synthesis is the increase in VO_{2max} induced by HIIT (23, 24). The intensity of exercise is one of the influential factors in improving the condition of mitochondrial biogenesis in the elderly. Intense training, including HIIT, increases the AMP/ADP/ATP ratio, followed by a further increase in AMPK. This factor can further increase ERRα and PGC-1a (as an upstream factor) (18, 25). Green et al. (26) examined the metabolic stress during exercise in a steady state following ten days of 30 or 60 minutes of cycling at a low, medium, and high intensity (60-86% VO_{2max}). Their study showed that following higher-intensity exercise, the cumulative levels of AMP and ADP and depletion of phosphocreatine occur in the muscle. Recently, a study has shown that increasing the volume of HIIT exercise (with increasing frequency and duration) increases the muscles' mitochondrial content (10).

Therefore, exercise frequency is also one of the effective factors in increasing mitochondrial biogenesis (18). Other factors appear to be involved in increasing AMPK expression. Furthermore, indicators such as SIRT1 and p53 as upstream factors can affect AMPK activity and expression. PGC-1α can also directly induce DNA to transcribe P53 (27). It seems that Short-term muscle activity can lead to P53 activation by increasing AMPK and P38MAPK levels.

Nevertheless, the intensity of exercise has been shown to be an essential factor in increasing p53 expression in order to increase PGC-1α expression. In such a way that only periodic rapid exercise increased p53 (25). So it seems that the intensity of exercise training is an important factor in increasing p53 expression, which has not been measured in this study to justify higher expression levels of PGC-1α compared to CT.

Conclusion

HIIT appears to improve mitochondrial function by increasing PGC-1α and ERRα levels. Probably one of the reasons for the increase in mitochondrial proteins in the gastrocnemius muscles is the training nature of HIIT (short and severe episodes). Considering the favorable effects of HIIT training on mitochondrial biogenesis compared to continuous training, it is suggested to use HIIT training to improve muscle performance in the elderly. However, considering the effect of IGF-1/Akt in inhibiting muscle atrophy and their impact on MAPK activation, it seems that the lack of evaluation of IGF-1/Akt/mTOR pathway is one of the limitations of this study. Therefore, studying hypertrophy signaling pathways under the influence of type and intensity of exercise in sarcopenia should be considered in future studies. Also, ATP and ADP are known as mitochondrial function indices, so in this study, the lack of measurement of ADP/ATP ratio is another limitation of the present study. Therefore, it is suggested to evaluate more physiological and pathological parameters of mitochondria in future studies.

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Authors' Contributions

H.P., A.B.; Contributed to the conception and design, to all experimental work, data and statistical analysis, and interpretation of data. H.P.; Was responsible for overall supervision. B.A., O.R.S.; Drafted the manuscript, which was revised by H.P., A.B., B.A., OR.S. All authors read and approved the final manuscript.

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