Research Article

The effect of eight weeks of interval and continuous endurance training on PGC-1α and FNDC5 gene expression in the gastrocnemius muscle of male Wistar rats

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Abstract

The purpose of this study was to investigate and compare the effect of eight weeks of continuous and interval training on PGC-1α and FNDC5 gene expression in the gastrocnemius muscle of male Wistar rats. 30 eight-week-old rats with an average weight of 192 ± 10 were randomly divided into 3 groups (n=10): control group (Ctrl), interval endurance training group (IET) and continuous endurance training group (CET). The exercise groups participated in 8 weeks of continuous or interval training program (5 days per week). 24 hours after the last training session, all the rats were anesthetized and their gastrocnemius muscles were collected and transferred to the laboratory to measure the mRNA levels of PGC-1α and FNDC5 genes by Real Time-PCR method. The results showed that the expression level of FNDC5 in the gastrocnemius muscle was significantly higher in the interval and continuous endurance training groups than in the control group. Also, the expression of this gene was higher in the interval group than in the continuous group. It was also found that the expression of PGC-1α gene was significantly increased in both training groups compared to the control group. Also, the expression of this gene increased significantly in the interval training group compared to the continuous. It seems that interval endurance training has a greater effect on the expression of PGC-1α and FNDC5 factors in the gastrocnemius muscle.

Key Words: Interval endurance training, Continuous endurance training, Gastrocnemius muscle, PGC-1α, FNDC5

Introduction

Today’s industrial life has caused people to stay away from physical activity, and this factor leads to physical poverty and obesity. During the recent years, the prevalence of obesity and overweight has grown a lot and now it has become a serious problem in the world (Rosiek et al., 2015). Obesity plays a negative role in health mammals. It has also been stated in recent research that acute and chronic exercise induces a brown adipose tissue phenotype to white adipose tissue through a number of key molecular factor. The results also show that regular physical activity will increase the expression of peroxisome proliferation activator alpha gene (PGC-1α) in human skeletal muscle. PGC-1α is a transcription regulator that can facilitate multiple transcription factors to regulate the complex network of genes. So that it plays a role in controlling tissue mitochondrial content and the program that leads to the formation of brown fat tissue (Bostrom, 2012; Cannon & Nedergaard, 2004; Lshibashi & Seale, 2010; Norrbom et al., 2004). Recently, a study has shown that exercise training causes the expression of PGC-1α in skeletal muscle, which can regulate the conversion of fibronectin type III domain containing (FNDC5) to irisin (Bostrom, 2012). It has also been stated that the expression level of FNDC5 was increased in the muscles of transgenic mice that overexpressed PGC-1α and in mice that performed endurance training (Maak et al., 2021).

Although PGC-1α plays an important role in regulating cellular energy metabolism, it is most likely involved in metabolic disorders such as obesity, diabetes, and cardiomyopathy. However, how it relates and affects the expression of FNDC5 is unclear, especially during exercise training (Dong et al., 2018; Liang & Ward, 2006). For this reason, the aim of this study was to considering the effect of eight weeks of continuous and interval training on the expression of PGC-1α and FNDC5 genes in the gastrocnemius muscle of male Wistar rats.

Materials and Methods

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Ethics statement

The investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996) and the professional governmental guidelines, in compliance with the Institutional Animal Care and Use Committee (IACUC) at Azad University (Science and Research Branch Tehran, Iran), were complied with in all experiments.

Animals

Thirty 8-week-old male Wistar rats with an average weight of 192 ± 10 grams were purchased from Pasteur Institute, Tehran, Iran. The rats were kept in the animal laboratory under controlled conditions of light to dark (12/12), light start at 6 o'clock in the morning and turn off at 6 o'clock in the evening, temperature (22 ± 3 C), and humidity (about 45%). Three to five rats were kept in Plexiglas cages with mesh lids, measuring 25 x 27 x 43 cm. All animals had free access to food and water. The animals were randomly divided into three groups: control group (Ctrl), interval endurance training group (IET) and continuous endurance training group (CET).

After 48 hours of adapting the rats to the environment and keeping conditions in the laboratory, they were exposed to the treadmill for one week in order to familiarize them with the exercise training. The familiarization program for all rats included 5 sessions of walking on the treadmill at a speed of 10 m/min and a 0-degree slope for 10 minutes. Also, the training groups participated in 8 weeks of continuous or interval training program (Figure 1).

Exercise training programs

The rats in the continuous endurance training group in this study started running on a rodent treadmill for eight weeks (5 days a week) at a speed of 10 m/min and for 20 minutes in each session, which gradually increased to a speed of 15 m/min and a duration of 60 min at end week.

The rats of the interval endurance training group performed eight weeks of interval training (5 days a week) in the form of a 6-minute warm-up at a speed of 10 meters per minute, 3 intervals of 4 minutes at a speed of 20-30 m/min. Along with a 2-minute active rest at a speed of 10 m/min between high intensity exercise. Finally reached 5 repetitions at the end of the eighth week. A duration of 6 minutes with a speed of 10 m/min was also considered for cooling down (Jones, 2007).

Tissue Processing

After 48 hours from the last training session, the rats were anesthetized with a combination of ketamine (90 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection. After making sure that the animals are anesthetized and taking blood from the heart tissue, the gastrocnemius muscle was immediately extracted and washed in physiological serum. Then the extracted tissue was frozen in liquid nitrogen and kept in a freezer at -80 °C until the test.

Gene expression (Real time PCR)

Gene expression was measured by real time-PCR quantitative method using RealQ Plus 2x Master Mix Green of AMPLIQON company and using 250 ng concentration of cDNA in duplicate. The design of primers was done based on the information of PGCIα and GAPDH genes in NBCI gene bank and by the company (Pishgam, Iran). Amplification was considered in each PCR cycle at a temperature of 95 °C for 15 seconds and according to the annealing temperature of the primers, each cycle was considered for 30 seconds (40 cycles). GAPDH was used as a reference gene. The expression level of the desired genes was measured by the formula.

Statistical Analysis

Figure 1. Timing of exercises performed for experimental groups.
### Results

#### Body weight

Comparison of body weight of animals in different research groups (post-test) is shown in Table 2. As can be seen, performing 8 weeks of CET and IET caused a significant decrease in the body weight of the animals (p<0.01 for both). The highest weight loss was related to the IET group (Table 2).

#### PGC-1α mRNA

The changes in the PGC-1α mRNA in the gastrocnemius muscle are shown in figure 2. Results shows that there is a significant difference between the gene expression of PGC-1α in the gastrocnemius muscle between the three interval endurance training groups, the continuous endurance training group and the control group (F=38.894, p = 0.001). Also, Tukey's post hoc test shows that there is a significant difference between the PGC-1α mRNA at gastrocnemius muscle in the interval endurance training group and the continuous endurance training group compare to the control group (p<0.05).

#### FNDC5 mRNA

The changes in the FNDC5 mRNA at gastrocnemius muscle are shown in figure 3. There is a significant difference between the FNDC5 mRNA in the gastrocnemius muscle between the three interval endurance training groups, the continuous endurance training group and the control group (F=126.342, p<0.001). The results of Tukey's post hoc test shows that there is a significant difference between the gene expression of FNDC5 in the gastrocnemius muscle in the interval endurance training group and continuous endurance training group compare to the control group (p ≥ 0.001). Also the expression of FNDC5 mRNA was higher at interval endurance training group compare to continuous endurance training group (p<0.001).

### Discussion

In the present study, the effect of eight weeks of continuous and interval training on the expression of PGC-1α and FNDC5 RNA in the gastrocnemius muscle of Wistar rats was investigated. The obtained results showed that interval and continuous endurance training increases the mRNA levels of PGC-1α and FNDC5 genes in the gastrocnemius muscle of rats. In this regard,
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In another study (Khalafi et al., 2020), the increase in PGC-1α gene expression stimulated the increase in FNDC5 expression (Khalafi et al., 2020). The upstream signaling pathway for PGC-1α activation is not fully understood, but appears to be ADP/AMP-ATP intracellular changes are the reason for this activation (Chen et al., 2000). However, the present study has many limitations such as the measurement of irisin level as well as the upstream and downstream pathways of PGC-1α and FNDC5. However, due to the important role of exercise on the level of PGC-1α expression and its effect on FNDC5, extensive research is needed in the future.

**Conclusion**

It seems that interval and continuous endurance training in the gastrocnemius muscle of Wistar rats increases the expression of PGC-1α mRNA and subsequently FNDC5 mRNA. However, this effect is greater in interval training. However, more studies are still needed in this area.

**What is already known on this subject?**

Although PGC-1α plays an important role in regulating cellular energy metabolism, it is most likely involved in metabolic disorders such as obesity, diabetes, and cardiomyopathy.

**What this study adds?**

Interval endurance training in the gastrocnemius muscle of Wistar rats increases the expression of PGC-1α mRNA and subsequently FNDC5 mRNA more than continuous endurance training.

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**Compliance with ethical standards**

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

**Ethical approval** The investigation was conducted in accordance...
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**Informed consent** Animal study.

**Author contributions**

Conceptualization: SH.F.F., M.GH.; Methodology: M.S., M.Z.; Software: M.GH, M.S.; Validation: M.Z, SH.F.F.; Formal analysis: SH.F.F, M.S.; Investigation: M.GH, M.Z.; Resources: M.Z; Data curation: M.S; Writing - original draft: SH.F.F.; Writing - review & editing: M.S, M.GH; Visualization: M.Z; Supervision: M.GH; Project administration: SH.F.F; Funding acquisition: SH.F.F.

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