

Comparing the Effect of Aerobic Exercise with Different Intensities on FNDC5 Protein Level of Muscle Tissue in Obese Wistar Rats

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Abstract

Introduction: A recent over expression model demonstrated that peroxisome proliferator-activated receptor coactivator 1-alpha (PGC-1 α) regulates the expression of fibronectin type III domain containing protein 5 (FNDC5); a novel myokine with a potential role in stimulating brown-fat-like development in white adipose tissue. The aim of this study was to investigate the effects of eight weeks of aerobic training with different intensities on FNDC5 protein in soleus muscle tissue of obese male Wistar rats.

Methods: 24 adult rats (weight: 250 to 300 gr, BMI >30g/cm²) were divided into three groups: aerobic training with 70 to 75% Vo_{2max} (moderate intensity), aerobic training with 80 to 85% Vo_{2max} (high intensity) and, control group. All training groups carried out exercise training for 8 weeks running on treadmill (5 sessions/per-week for 60 min per session). After the training period, the level of FNDC5 protein was measured. Statistical test of ANOVA was used for data analysis to determine the difference between groups and post hoc test of Tukey was used for paired comparisons (p \leq 0.05).

Results: The levels of FNDC5 in soleus muscle tissue in both moderate intensity and high intensity aerobic training group increased significantly (P=0.001).

Conclusion: According to the results, increasing of FNDC5 as a result of eight weeks of moderate and high intensity aerobic training with moderate and high intensity, it seems that FNDC5 can mediate conversion of white to brown tissue and so affect losing weight and thermogenesis.

Keywords: FNDC5, Training, Muscle, Obesity

Introduction

Skeletal muscle as an endocrine organ can act as factor to release Myokines from the muscle. Various studies have shown that exercise and physical activity increase the expression of PGC-1 α . Recently, Boström *et al.* have shown that stimulating expression of PGC-1 α can activate several genes produced in the muscle, such as the irisin protein (encoded by the FNDC5 gene present in the skeletal muscle), which is found in the plasma of mice and humans after exercise (1). Over expression of FNDC5 in mice lead to 3 to 4 fold increasing of irisin in the plasma, which is due to browning of the subcutaneous fat and

thermogenesis (2). The FNDC5 protein (or PeP, also called FRCP2) is derived from one signal peptide, one fibronectin branch of type III, and one C- terminal hydrophobic branch (3). The FNDC5 ultra-membrane type with molecular weight of 32 kDa is larger than its membrane type. This difference creates the hypothesis that FNDC5 splits in its c-terminal region before its secretion (1). The expression of FNDC5 gene can be enhanced by PPAR γ and PGC-1 α mediators. The FNDC5 gene is a factor to encode the type 1 membrane protein. This action increases with muscle activity and affects the process of making irisin. The irisin cleavage and release process is similar to other

membrane polypeptides, including the Epidermal Growth Factor (EGF) and Alpha Transformation Growth Factor (TGF α)(4). Following the loss of C-terminal signal peptide, C-terminal peptide becomes two halves and glycosylated. Then it is released as hormone with 112 amino acids. In mammals, the irisin sequence that results from the breakdown of FNDC5 remains almost intact. Studies have shown that this structure is the same in humans and mice (1). Briefly, there is a hypothesis that the amount of FNDC5 protein in the muscle increases as a result of the exercise. Subsequently, some irisin is released and by effecting the brown and white adipose tissue, leads to increased energy consumption and, finally, weight loss (5). Irisin induces UCP1 gene expression after binding to its membrane receptor in white adipocytes and through this, it is effective in converting white adipose tissue into the Brite adipose tissue and increasing the cost of total energy, weight loss and improving insulin sensitivity (1, 6). However, in this regard, Reisi *et al.* (2013) investigated the acute effect of resistance exercises (exercises were performed three times in a week, with five repetitions, three-minutes of rest between turns, one-minute rest between repetitions) on plasma irisin protein and FNDC5 gene expression of muscle and UCP1 in abdominal adipose tissue on the 16 rats. They concluded that after a resistance exercises session, plasma irisin protein levels, the relative expression of mRNA in FNDC5 and UCP1 genes increased significantly (7). Jafari *et al.* (2015) examined the effect of eight weeks of aerobic exercises with three sessions per week and 30 minutes per session on 42 obese mice. They concluded that the level of FNDC5 in muscle and heart tissue of mice increased, but it was not statistically significant (8). In contrast, Bonafiglia *et al.* (2017) examined the effect of aerobic exercises in different ways such as continuous periodic exercises, intense periodic exercises and periodic-speed exercises on PGC-1 α mRNA expression and FNDC5

mRNA expression. They concluded that in all three groups, PGC-1 α mRNA expression was significantly increased, but no significant changes were obtained in FNDC5 mRNA (9). Timmons and colleagues (2012) reported that, there was no evidence of increased FNDC5 expression after exercise in humans. At the same time, they showed that the expression of FNDC5 in older participants was 30% higher than active sedentary control subjects(10). In sum, there is convincing evidence that exercises can act as an activator of brown adipose tissue in the prevention and treatment of obesity. But the available studies included contradictory results. In some studies, a significant or non-significant relationship has been reported among the effect of physical activity and exercise indicators (8, 9, 11, 12). There are few studies about the effect of physical activity and exercise on changes in fatty tissue phenotype. So that, researchers have not been able to achieve significant consensus of improvement health-related adaptations in order to make a relationship between type, intensity and volume of exercises with FNDC5 values. Currently, it is hypothesized that the change in intensity and volume of exercise, acute responses and adjustment of this hormone is different. According to researches, aerobic exercise is one of the factors affecting the secretion of FNDC5, therefore, considering the relatively new hypothesis of changing the fatty tissue phenotype from white fat to brown fat and its effect on weight loss and, on the other hand, the correlation between the weight changes taken from FNDC5 changes and can be a factor in improving the regulation of homeostasis in the body and improving insulin sensitivity, it would seem reasonable that activities with aerobic nature could alter the level of the FNDC5 protein. Although, FNDC5 is one of the factors that can activate brown adipose tissue and have anti-obesity effects, there have not been done any research that focused on simultaneously examining the different intensities of aerobic exercise on

FNDC5 changes. Therefore, the purpose of this study was to study the comparison the effect of aerobic exercise with different intensities on FNDC5 levels in male Wistar rats.

Methods

In this experimental study twenty-four healthy male Wistar rats (eight-week-old rats, weighing 250-300 gr, body mass index more than 30 g/cm²) were used. The rats were purchased from the Iran Razi serum Institute and transferred to the animal lab at Faculty of Physical Education and Sports Sciences of Ferdowsi University of Mashhad. The rats were kept for 2 weeks in a cage made of polycarbonate under controlled conditions, i.e. average ambient temperature 23±1 ° C, humidity 50 ± 3% and 12-h light/12-h dark cycles with free access to an ad libitum food and water. Water was provided to rats by special glass bottle. Cages were disinfected 3 times a week with 70% alcohol. All animals had free access to water and special food for rats. After two weeks of familiarity with the laboratory environment, the animals were randomly divided into three groups: moderate intensity aerobic exercises (n=8), high intensity aerobic exercises (n=8) and control group (n=8). It should be noted that in all stages of the research, the principles of the Helsinki Declaration and the opinions of ethics committee research were observed. Also, for the research project, the No. 131 license of the research ethics committee was obtained from Ferdowsi University of Mashhad. After 48 hours from the last exercise session and 12 hours fasting, rats of all groups were scarified after transferring to the laboratory of the Faculty Pharmacy in Ferdowsi University of Mashhad. In the first phase, the animals were placed in sampling special space (sterile environment) and anesthetized with combination of ketamine (30-50 milligrams per kilogram body weight) and xylazine (3-5 milligrams per kilogram body weight). Anesthesia of the rats was confirmed by

assessing toe reflexes. Then, 5 to 6 cm long cut was made in the abdominal region of rat body, and the soleus muscle were quickly removed, washed in normal saline solution, and transferred to a 1.5 ml micro tube and placed immediately in liquid nitrogen and was kept at in a freezer at -80°C for further analysis (13). After the tissue samples were removed from the freezer, liquid nitrogen was added and was pounded by pestle and mortar. Then, the obtaining powder was transferred to the homogenizer tube and combined with RIPA buffer solution. After that, the sample was mixed for 15 seconds by homogenizer machine (Potter Elvehjem). All steps were carried out inside the ice container. At the last step, the specimen was drawn from the tube by sampler and poured into 1.5 ml microtube. The micro-tube was centrifuged at 4 ° C for 20 minutes, at 20,000 rpm (RPM). After completion of the centrifuge, the micro-tubes were removed from the device; also supernatants were removed by sampler and poured into new micro tube one. Then the samples were stored at -80 ° C. The level of FNDC5 protein was measured with ELISA Kit for Rat (EASTBIOPHARM, China, under license from the US) With Cat.No: CK-E91393 serial number and Intra-Assay Sensitivity: CV <10%. According to Table 1, moderate and high intensity aerobic exercise program performed for eight weeks, five days a week, and one session per day for 60 minutes between 08:00 and 12:00 in the morning at different speeds on rodent treadmill. After adaptation was completed, and during the first week, animals (on how to implement the aerobic exercise protocol) were placed on the treadmill and walked at speed of 10 m / min with zero-degree gradient for 15 minutes. During the second and third weeks, the speed and duration of the exercise gradually increased, so that the speed of running in the moderate intensity group on the treadmill was 28 m / min and equivalent to 70 to 75% Vo₂max. Also, the speed of running in the high intensity group was 34 m / min and

equivalent to 80 to 85% Vo_{2max} . In sum, the exercise volume in moderate intensity groups was 8.4 km per week and in high intensity exercise was 10.2 km per week (14-16). After completing the exercise program, the speed of the device declined inversely to zero for cooling down the body. The data were analyzed by SPSS software version 16. The normal distribution of the data was assessed using the Shapiro-Wilk test and homogeneity of variances by Levene's test. Then one-way ANOVA and Tukey post hoc tests were used to examine the between group differences. Moreover, Pearson correlation was used to determine the relationship between the variables. Also, the decision criterion for accepting or not accepting hypotheses was considered at the level of $P \leq 0.05$.

Results

According to Table 2, intra-group differences in mean body weight in control group increased significantly ($p = 0.001$) and decreased significantly in moderate and high intensity exercise groups ($p = 0.001$). Based on the results of Tukey's post hoc test, there was a significant difference between the eight weeks

of aerobic exercise with moderate intensity with the control group ($p = 0.001$) and the high intensity group with the control group ($p=0.001$). According to Table 3, there was a significant difference between the FNDC5 protein content of muscle tissue in male Wistar rats in moderate intensity aerobic exercise group and in high intensity aerobic exercise group ($F=12.49$ and $P=0.001$). Based on Tukey post hoc test, there was significant difference in FNDC5 protein concentration at muscle tissue between two groups of high intensity exercises and control group ($p=0.04$). Also, there was significant difference in FNDC5 protein concentration between the two groups of moderate and control groups ($p=0.02$) (Table 3). The results of Pearson correlation test showed that in high intensity aerobic exercise group there was significant correlation between body weight and FNDC5 protein level ($p=0.04$) nevertheless in moderate intensity aerobic exercise ($P= 0.94$) and control groups ($P = 0.96$) there were no significant relationship between weight and FNDC5 protein level.

Table 1. Program exercise with moderate and high intensities

Weeks	Moderate intensity	High intensity
First	15 min/speed 10 m.min	15 min/speed 10 m.min
Second	27 min/speed 15 m.min	27 min/speed 15 m.min
Third	34 min/speed 20 m.min	35 min/speed 20 m.min
Fourth	40 min/speed 21 m.min	45 min/speed 22 m.min
Fifth	46 min/speed 23 m.min	54 min/speed 24 m.min
Sixth	52 min/speed 24 m.min	59 min/speed 27 m.min
Seventh	58 min/speed 26 m.min	60 min/speed 31 m.min
Eighth	60 min/speed 28 m.min	60 min/speed 34 m.min

Table 2. The variation of weight in experimental and control groups after eight weeks of aerobic exercise.

Variable	Time	Control (M±SD)	Moderate intensity aerobic exercise (M±SD)	High-intensity aerobic exercise (M±SD)
weight (g)	Pre- test	296.25±1.65	296.08±1.56	294.50±1.97
	Post- test	302.58±1.16	287.41±4.25	287.08±4.23

Table 3. The variation of FNDC5 proteins in experimental and control groups after eight weeks of aerobic exercise

Variable	Control (M±SD)	Moderate intensity aerobic exercise (M±SD)	High-intensity aerobic exercise (M±SD)
FNDC5 (ng/ml)	39.56±1.72	48.24±6.68*	41.85±1.87*

*Significant difference with control groups at 0.05

Discussion

The aim of this study was to compare the effect of aerobic exercise with different intensities on FNDC5 protein level of muscle tissue in obese Wistar rats. Based on the obtained results of the present study, the moderate and high intensity aerobic exercise lead to a significant increase in FNDC5 protein content of muscle tissue in obese Wistar rats. This finding is consistent with the results of Liu *et al.* (2015) (17), While are not consistent with the results of Jafari *et al.* (2015) Czarkowska *et al.* (2013) and Pekkala *et al.* (2013) (8, 18, 19). Liu *et al.* (2015) randomly divided 40 mice into four control groups, half-hour exercise session, one-hour exercise group, 1.5-hour exercise group, and two-hour exercise group. They examined the effects of high intensity exercises over treadmill on irisin expression and FNDC5 expression changes, ACC β and concluded that by sudden increasing the intensity of exercise, the content of FNDC5 protein and the P-ACC β / ACC β ratio increased significantly (17). By contrast, Jafari *et al.* (2015) studied the effect of eight weeks, three sessions per week, and 30 minutes during each aerobic exercise. They found that need rewording (8). Czarkowska *et al.* (2014) examined the effect of one exercise session during six weeks of increasing intensity aerobic exercise on 60 mice. They concluded that there was no significant change in the level of FNDC5 (19). Pekkala *et al.* (2013) studied the effect of four types of exercises; one hour of low-intensity aerobic exercise, one hour of high-intensity resistance exercise, 21 weeks of long-term aerobic exercise and combined exercise (long-term exercise + resistance exercise) on irisin and

expression levels FNDC5 mRNA. They concluded that there was no significant change in PGC-1 α , FNDC5, skeletal muscle and serum irisin in low intensity aerobic exercise groups, 21 weeks of long-term aerobic exercise and combination exercises (long-term exercise + resistance exercise)(18). The expression of FNDC5 gene increases through PPAR γ and PGC-1 α mediation. The FNDC5 gene is a factor of coding the type 1 membrane protein (UCP1), this action increases with muscular activity and affects the process of making irisin (1). Also, the FNDC5 protein upregulate the other genes in brown adipose tissue, such as Elov13, Cytochrome c oxidase polypeptide 7A1 (Cox7A) and Otopettrin 1 (Otop1), but it down regulates leptin, which is a product of the white adipose tissue(1). According to the studies, 20 nmol of FNDC5 protein can increase the UCP1 gene by 7 to 1500 times. UCP1 has the ability to reduce the amount of proton produced by oxidative phosphorylation. This protein is called Thermogenin and known as separating agent. By increasing permeability, the mitochondrial membrane allows protons to be pumped into the membrane and return to the mitochondrial matrix. As a result, the electrochemical potential is reduced and ATP synthesis is not performed. This is due to the fact that the energy is converted to heat despite the speed of the electron transfer chain (20). Muscle activity leads to increase calcium ion from the sarcoplasmic network, and then calcineurin and calmodulin kinases are activated. These factors producing PGC1 α in the nucleus due to the effects on CREP transcription factors, Nuclear factor of activated T-cells (NFAT) and Myocyte-specific enhancer factors C & D

(MEF) (21). On the other hand, increasing calcium ion affects AMPK and activates PGC1a and thus the FNDC5 protein is transcribed inside the nucleus. FNDC5 is broken down by proteolysis and released as an irisin hormone in the blood. After reaching irisin into target cells, UCP1 is increased and it converts white fat into brown fat (21). According to the research, intensity of exercise is one of the main factors influencing changes in the fatty tissue phenotype. Physical activity intensity is one of the discussed effects of physical activity on changes in white adipose tissue phenotype to brown adipose tissue. Of course, when comparing two intensities of aerobic exercise, intensive and moderate aerobic exercises both increase FNDC5 levels. In general, according to the results of this research, making changes in the factors involved in the exercise protocol, such as intensity of exercise, the duration of exercise, the nature and type of exercise, the frequency of repetitions per week and the duration of each training session can be investigated.

Conclusion

The results of the present study show that both types of aerobic exercise with different intensities can have positive effect on changes in FNDC5 levels. It seems that irisin is affected by a variety of sports activities, plays a role in the conversion of white to brown adipose tissue and can contribute to weight loss and increased thermogenesis. However, the irisin response to a variety of sports activities with different intensities is still unknown and requires further research.

Ethical issues

Not applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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