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Research Article

Effect of resistance training and nanocurcumin supplementation on the expression of FNDC5 and PPARɣ genes in rat muscle tissue

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Abstract

Background: Irisin is released from the Fndc5 protein in muscle cell through physical activity and effects on metabolism through browning of white fat. The purpose of this study was to the effect of resistance training and supplementation of nanocurcumin on the expression of genes of FNDC5 and PPARɣ rat muscle tissue.
Materials and Methods: In this experimental study, 32 rats were randomly divided into four groups (Control, resistance training, nanocurcumin, resistance training + nanocurcumin). The training groups program included 4 weeks, 3 days a week from climbing on a stepladder. Nanocarcmine (80 mg / kg) was given gavage in complementary groups for four weeks daily. FNDC5 and PPARɣ gene expression were measured using the RT-PCR method. Data were analyzed using one-way ANOVA with a significant level of P≤0.05.
Results: The results showed that resistance training and supplementation of Nanocarcmine significantly increased the expression of the gene of FNDC5 and PPARɣ in muscle tissue of rat (P <0.05).
Conclusion: It seems resistance training with nanocurcumin supplementation may stimulate secretion of FNDC5 & PPARɣ from muscle, that has a key role in the metabolism of adipose tissue and the conversion of white tissue to brown fat tissue.

Keywords:
Resistance training, Nanocurcumin, FNDC5, PPARɣ

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1. Introduction

Adipose tissue of the endocrine glands is a prominent link between obesity and metabolic diseases by producing some important factors called adipokines. (1). There are two types of adipose tissue, including white adipose tissue and brown adipose tissue in mammals. White adipose tissue is a source of energy storage and brown adipose tissue plays a role in increasing UCP-1 protein, heat production, energy consumption and weight regulation (2). Researchers have recently found that skeletal muscle plays an active role in regulating metabolic homeostasis through its ability to connect with adipose tissue and endocrine glands (3). Skeletal muscle is an active metabolic tissue whose contraction increases the release of several myokines, such as interleukins 6, 8, 10, and 15, leukemia inhibitory factor, fibroblast growth factor, brain-derived neurotrophic factor, and myokine called irisin, which are able to interact with adipose tissue. Irisin is a 112 amino acid protein that is secreted from skeletal muscle immediately after exercise (4). Exercise induces the expression of the PGC-1α gene in skeletal muscle, and PGC-1α stimulates the expression of the fibronectin type III gene containing the glycoprotein FNDC5 in muscle tissue (5). PGC-1α, on the other hand, is a coagulator for PPAR. PPAR is involved in energy metabolism, which in turn stimulates the expression of the FNDC5 gene (6). Hosseinzadeh et al. (2015) reported increased expression of FNDC5 twin muscle gene in male rats after eight weeks of endurance and resistance training (7). Han et al. (2017) investigated the effect of aerobic exercise on the expression of skeletal muscle FNDC5 gene due to high-fat diet. The results showed a positive effect of exercise with or without a high-fat diet on FNDC5 gene expression (8).

However, Hashemi et al. (2015) stated that 4 weeks of HIIT training had no effect on the protein content of PPARɣ subcutaneous adipose tissue in obese diabetic rats (9). The possible effects of herbal medicines have been confirmed by several studies (10). Curcuminoids are one of the most important anti-inflammatory substances in nature (11). Curcumin has significant functional properties including anti-tumor and anti-cancer activities, lowering blood and liver cholesterol levels, enhancing immune function, inhibiting cardiovascular disease, anti-inflammatory, protection against Alzheimer's disease and antioxidant properties (12). Curcumin can increase UCP1 and other browning proteins such as PGC-1α, PRDM16 in white adipose tissue via the AMPK pathway and the norepinephrine-beta-adrenoceptor pathway (13). Modification of adipose tissue phenotype due to exercise and supplementation is a new theory that has recently been proposed and the identification of its molecular cellular mechanism is being investigated. A single study was not found that examined the simultaneous effect of resistance training and nanocurcumin supplementation on the expression of FNDC5 and PPARɣ genes in muscle tissue. Therefore, in the present study, the researcher intends to investigate the effect of resistance training and nanocurcumin supplementation on the expression of FNDC5 and PPARɣ genes in rat muscle tissue.

2. Materials and Methods

The present study is experimental. In this study, 32 2-month-old male Wistar rats with an average weight of 200 to 220 g were purchased from the Pasteur Research Institute. Rats after transfer to the laboratory environment and familiarity with the new environment, were randomly divided into 4 groups (8 heads) control, nanocurcumin supplementation, resistance training, resistance training + nanocurcumin. Rats were kept in standard conditions of laboratory animals (temperature 25-23 ° C, humidity 40-50% and light-dark cycle 12:12). All rats had free access to standard laboratory animal feed as well as water. Ethical principles of the study, in accordance with the principles of working with laboratory animals approved by the Islamic Azad University, East Tehran Branch and receiving the code of ethics IR.IAU.ET.REC.1400.032 were observed by researchers during the research.

Resistance training program was performed using a ladder for rat resistance training. The familiarity stage was performed for a week to climb the ladder to a height of 1 meter and a slope of 85 degrees by tying a weight to the mouse tail. The training program was performed for 4 weeks in 3 sessions per week in the form of 3 sets with 4 repetitions in each set. Rest intervals between sets were 3 minutes, rest intervals between repetitions in each set were 30 to 60 seconds. Applying resistance by tying weights to the tails of mice was equivalent to different percentages of body weight during the training period. The details of the exercise program are summarized in Table 1 (14).

Table 1: Resistance training protocol based on body weight percentage

Training time	first week	second week	Third week	forth week
Exercise resistance (body weight percentage)	30%	50%	80%	**100%**

80 mg of nano-curcumin (commercial nano-curcumin made by Exir Nano Sina Company (Tehran, Iran) per kg of body weight (mg / kg) in groups Supplements and exercises + supplements were given by gavage (15). Due to the half-life of gene expression 48 hours after the last training session, the animals were first sampled in a special area (sterile environment) with a combination of ketamine (30 to 50 mg per kg of body weight) and xylazine (3.5 mg / kg body weight were anesthetized. Blood samples were then taken from the left ventricle at a rate of 5 cc and immediately poured into test tubes containing EDTA anticoagulant and stored at -20 ° C. After blood sampling, soleus muscle tissue was separated through a slit on the lateral dorsal region of the lower limb and after weighing was placed in liquid nitrogen, then transferred to a freezer at -80 ° C. In order to extract mRNA, 50 mg of frozen tissue was used by homogenization method. For mRNA isolation, the German Qiagen kit was used according to the manufacturer's instructions.

The extracted RNA solution was cleaned of any DNA contamination and RNA-degrading enzymes using the German Qiagen kit. From the sample, two micrograms of mRNA was used to synthesize the first cDNA strand. CDNA synthesis was performed with the transcription first strand cDNA synthesis kit (Roche, Germany) and according to the kit instructions. Finally, Real time PCR was performed using a device (Rotrogene 6000, Corbet). Real time PCR program for genes based on cybergreen from Qiagen (Germany) and includes: initial washing at 95 ° C for 10 minutes and 45 cycles including: washing at 95 ° C for five seconds, binding of primers at appropriate temperature 57 ° C for 20 seconds, expansion at 72 ° C for 15 seconds, adjustment of melting temperature in the range of 55 to 99 ° C to form a melting curve diagram. Melting Curve was also plotted to ensure the specificity of the reaction product. Internal control for FNDC5 and PPARɣ genes was performed by GAPDH household gene (Pishgam, Iran). Finally, data quantification was performed using the formula 2 - ^ (ΔΔ).

Table 2: Primers used in Real-time PCR

	Gene	sequence
1	Rattus norvegicus fibronectin type III domain containing 5 (**FNDC5**)	Forward: AGGACCTCACTGTTCTGACG
		Reverse: AGGGGTTAGTTGGAGGCTTC
2	Rattus norvegicus peroxisome proliferator-activated receptor gamma (**PPARɣ**)	Forward: ATCCCGTTCACAAGAGCTGA
		Reverse: GCAGGCTCTACTTTGATCGC
3	**GAPDH**	Forward: CAAGTTCAAGGGCACAGTCA
		Reverse: CCCCATTTGATGTTAGCGGG

Kolmogorov-Smirnov test was used for normal distribution of data and Levene test was used to check the homogeneity of variances. To compare the differences between the groups, one-way analysis of variance was used and then Bonferroni test was used. Statistically significant difference was determined at the level (P≥0.05). SPSS software version 25 was also used for data analysis.

3. Results

Data analysis showed that there was a significant difference in the rate of changes in FNDC5 gene expression in muscle tissue between different groups (P <001) (Table 3). The results of Bonferroni post hoc test showed that there was a significant difference between the control groups and the resistance training group, nanocurcumin and nanocurcumin + resistance training (P <001). (Figure 1). Other results of the present study, there is a significant difference in the amount of changes in the expression of PPARɣ gene in muscle tissue between different groups (P <001) (Table 3). The results of Bonferroni post hoc test showed that there was a significant difference between the control groups and the resistance training group, nanocurcumin and nanocurcumin + resistance training (P <001). (Figure 2).

Table 3: Results of one-way analysis of variance FNDC5 and PPARɣ in four research groups

Variable		**Sum of squares**	**df**	**F**	**Sig**
FNDC5	Between groups	2. 485	3	67.77	0.000
	Within the group	0. 342	28		
PPARɣ	Between groups	397.197	3	24. 09	0.000
	Within the group	477.76	28		

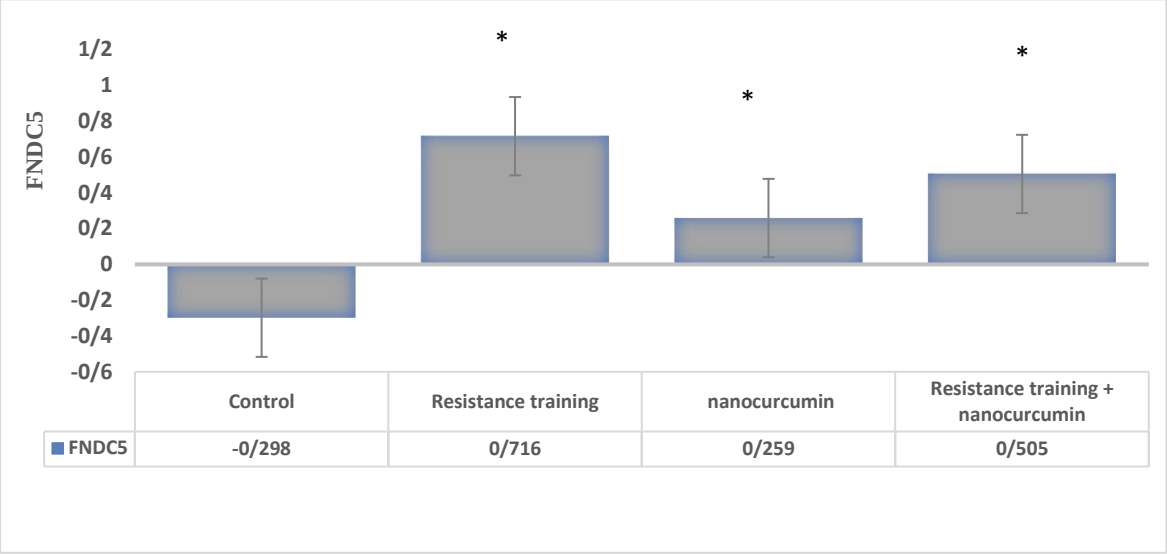

Figure 1: Comparison of mean FNDC5 gene expression in four groups: control, supplement, exercise, exercise + supplement.

*** Significant level P<0.05**

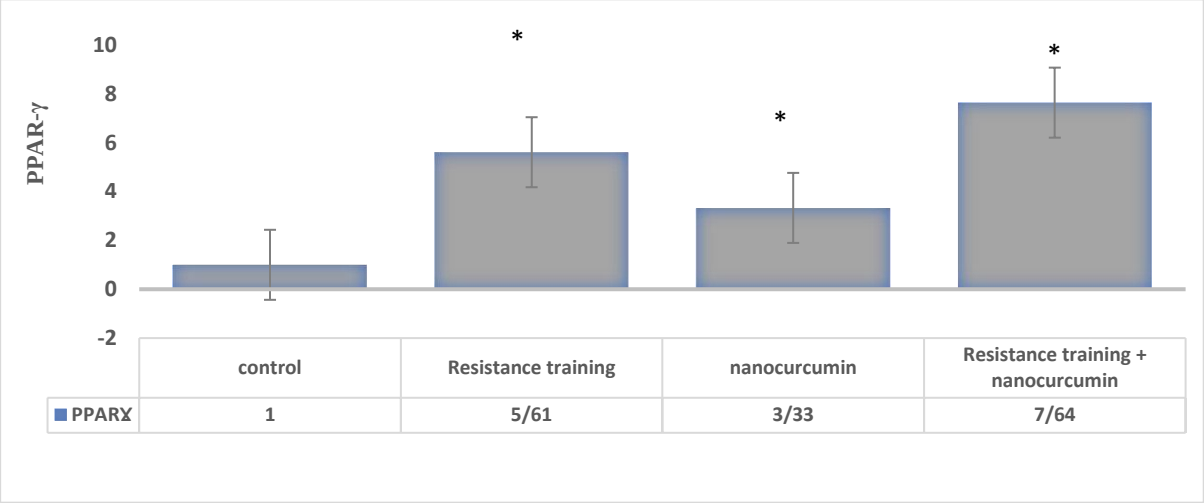

Figure 2: Comparison of mean PPARɣ gene expression in four groups: control, supplement, exercise, exercise + supplement.

*** Significant level P<0.05**

4. Discussion

In the present study, it was shown that resistance training and nanocurcumin consumption significantly increased FNDC5 gene in muscle tissue. PGC-1α is secreted into skeletal muscle following exercise, which completes the FNDC5 gene expression process. FNDC5 is then subdivided into a new form of irisin (9). During the 24-hour recovery phase, FNDC5 mRNA expression of protein and muscle tissue is maintained at a high level. The results show the coordinated expression of FNDC5 and PGC-1α in the increase of serum irisin levels after exercise (16). (16). Research has shown that irisin browns subcutaneous adipose tissue and increases energy costs and fat oxidation. Therefore, irisin is known as a new agent for the prevention and treatment of obesity (17). (17). The signaling pathway of PGC-1α expression and conversion of FNDC5 to irisin is activated through exercise (16). Khalfi et al. (2015) showed that high-intensity intermittent training significantly increased FNDC5 gene expression in diabetic male rats (18). (18). Ghaderi et al. (2017) stated that 14 weeks of endurance training with two different intensities significantly increased the expression of skeletal muscle FNDC5 gene in obese mice (19). However, Pang et al. (2018) reported that running on a rodent treadmill had no significant effect on serum levels of irisin, PGC-1α and FNDC5 in rats (17). These results are inconsistent with the results of the present study. Non-heterogeneity can be referred to the type of tissue evaluated, the species under study, the characteristics of the exercise practiced, the characteristics of the subjects under study and the measurement methods. Studies have shown that resistance training with a high number of repetitions in the muscle group can lead to increased muscle endurance and muscle group.

The metabolic effects (muscle growth) of resistance training appear to be associated with increased FNDC5 expression. Previous studies have shown increased muscle strength, mTOR signaling, and hypertrophy in response to resistance training (20). As a result of these exercises, different signals activate PGC-1α in skeletal muscle, which can subsequently stimulate the expression of FNDC5 and act on white adipose tissue in the long run by secreting irisin-induced FNDC5 into the bloodstream. Increases UCP1, which indicates an increase in calorific value and energy cost through its conversion into heat (21). Curcumin can also increase UCP1 and other browning proteins such as PGC-1α, PRDM16 in white adipose tissue via the AMPK pathway and the norepinephrine-beta-3-adrenoceptor pathway (22). A recent study reported that combination therapy with endurance training and curcumin increased AMPK phosphorylation, NAD / NADH ratio, PGC-1α distillation, CAMP levels, and PKA downstream targets. The combination of curcumin and exercise accelerates mitochondrial biogenesis by increasing CAMP levels in skeletal muscle (23). Zou et al. (2021) stated in a study that the improvement in basal metabolic rate by curcumin may be partially regulated by the FNDC5 / p38 mitogen-activated protein kinase (p38 MAPK) / extracellular signal-related kinase (ERK) pathway 1/2 (24). Another result showed that resistance training and nanocurcumin supplementation significantly increased PPARɣ gene expression in muscle tissue of healthy male rats. Shabani and Darianush (2018) reported that eight weeks of LICT training, repeated 4 days a week, increases the protein content of PPARɣ in the adipose tissue of overweight diabetic mice and is one of the mechanisms for converting white fat to brown through protein mediation. Because the protein has a positive regulatory region of 16

Turgut et al. (2018) reported a significant increase in PPARɣ gene expression in rat liver and muscle after running on a treadmill for 6 weeks / 5 days a week (26). Various PPAR isoforms, such as PPARβ, PPARɣ, PPAR-α, mediate the beneficial effects of exercise. Gene expression and protein levels of these factors increase as a result of exercise, and the greater the intensity and duration of exercise, the greater the response or adaptation.

There are various mechanisms for increasing gene expression of these factors after exercise. Natural PPARs ligands include unsaturated fatty acids, 15-deoxy-D12, 14-prostaglandin G2 (15d-PGJ2), and 9-and-13-hydroxy-octacadionic acid (Hode-9, 13) (possibly increasing this). Factors after exercise can also increase PPARɣ gene expression. Fatty acids and eicosanoids can also increase the activity and expression of the PPARɣ gene. Gene expression, activity and interaction of PPARs with other transcription factors are among the determinants of PPARs signaling effects and one of the most important factors affecting these cases is the oxidative stress state of the cell (28). Increased exercise-induced mitochondrial activity to produce energy leads to increased production of reactive oxygen species (ROS), or oxidative stress. Oxidative stress activates regulatory kinases with extracellular ERK signal, platelet-derived growth factor (PDGF), and signaling inositol 3-kinase / protein kinase B (PI3K / AKT), which stimulates the expression of PPARs, as a They are considered defense mechanisms. Increased lipid oxidation due to exercise not only increases oxidative stress, but also stimulates gene expression and PPARs activity (27). Curcumin also appears to activate the FNDC5 / irisin pathway in healthy male rats by increasing PGC-1α and UCP-1. The findings of the present study are consistent with the findings that show that curcumin is able to increase the expression of PGC-1α (29).

Curcumin is also able to enhance several metabolic exothermic genes, including UCP-1, BMP8b, SIRT1, PGC-1α, and PRDM-16 (29). However, Hashemi Takmili et al. (2015) in a study examined the effect of 4 weeks of HIIT (4 days per week) on PPARɣ protein content in subcutaneous adipose tissue of obese diabetic male rats and did not observe a significant change (9).). Hemmati Farsani et al. (2019) mentioned PPARɣ as an adipogenic agent in bone marrow mesenchymal stem cells and stated that 8 weeks of resistance training (5 days per week) did not affect the expression of this substance in the tibia marrow. Slow (30). Important factors of this contradiction with the findings of the present study can be the type of exercise, intensity and duration and type of supplement under study and measurement methods.

5. Conclusion

Due to the increase in muscle content of FNDC5 and PPARɣ following resistance training combined with the use of nanocurcumin in this study and the important roles of these two variables in adipose tissue metabolism. It seems that doing resistance training with nanocurcumin can be an important mechanism to reduce white adipose tissue and turn it into brown adipose tissue.

Compliance with ethical standards

Conflict of interest The authors of the article state that there is no conflict of interest in the present study.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

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Author contributions

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

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