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ORGINAL ARTICLE

Irisin/FNDC5 Gene Expression in Fast-Twitch Extensor Muscles of the Forelimb and Slow-Twitch Soleus Muscles of Male Wistar Rats Affected by Resistance, Endurance, and Concurrent Training: Comparison between Different Types of Training

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KEYWORDS

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ABSTRACT: Exercise can exert its beneficial effects through specific mediators. One of the organs that is most affected during exercise is the skeletal muscles. These muscles release various myokines, of which irisin is one of the major ones that has been recently identified. However, the way the irisin gene, FNDC5, responds to different exercise training has not yet been fully elucidated. This foundational research aimed to investigate the effects of three different types of exercise on FNDC5 gene expression in the muscles of rats. 40 healthy male Wistar rats were divided into four ten groups of control, resistance training, endurance training and concurrent training. Each group did their own specific exercises for 8 weeks, except for the control group, which did not have any specific activities. Finally, fast-twitch extensor muscles of the fingers and slow-twitch soleus muscles were tested to measure FNDC5 gene expression. The analysis results showed that FNDC5 gene was expressed in all groups with a significant difference ($P < 0.001$). The relative expression of FNDC5 gene was significantly increased in all training groups compared to the control group ($P < 0.001$). In addition, comparing exercise groups together, combined exercises showed the greatest effect and endurance exercise showed the least effect. In intra-group comparison, the expression of FNDC5 gene in forelimb and hindlimb muscles was not significantly different ($P > 0.05$). All trainings could increase the expression of FNDC5 gene compared to the control group, but combined, resistance and endurance exercises had the greatest effect, respectively.

1. Introduction

Physical inactivity and a sedentary lifestyle are associated with the onset of many chronic diseases, including obesity, diabetes mellitus, cardiovascular diseases, sarcopenia, osteoporosis, and cancer [1-3]. On the other forelimb, exercise is one of the best ways to maintain health and prevent these diseases [4]. Regular exercise has beneficial effects on metabolic health by directly transferring glucose, improving insulin sensitivity, enhancing endothelial function, and strengthening the nervous system, thus reducing risk factors such as blood glucose and lipid levels [5].

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The metabolic effects of exercise seem to be mediated through certain mechanisms [6].

Skeletal muscles are the largest organ in the body and make up approximately 40% of body weight. They play a crucial role in regulating metabolism [7]. In addition to their local effects on their own metabolism, recent findings show that skeletal muscles also act like endocrine organs by releasing hundreds of peptides into the bloodstream, known as myokines or muscle cytokines [7, 8]. The discovery of myokines has opened a new window to understanding the effects of exercise and has provided evidence that muscles can communicate with other organs such as bones, the liver, adipose tissue, and the brain [9].

One of the myokines released from skeletal muscles is irisin. It is also referred to as an adipomyokine, because it is partially produced in adipose tissue as well [10, 11]. One study found that low levels of irisin are associated with lower 12-month survival rates in patients with coronary artery disease [12]. Furthermore, serum levels of this myokine are significantly associated with obesity, metabolic syndrome, lean body mass, fat mass, and insulin resistance [13, 14]. Therefore, irisin can have broad metabolic effects. Irisin promotes the browning of white adipose tissue and stimulates thermogenesis [15]. Thus, irisin influences energy homeostasis and is one of the key regulators of fat metabolism [16]. In the liver, irisin induces glycogenesis by reducing gluconeogenesis and lipogenesis through GSK3, FOXO1, and SREBP2 pathways [6]. Additionally, irisin stimulates cellular metabolism in heart tissue, prevents cell proliferation, and enhances cell differentiation by activating PI3K-AKT and Ca^{2+} signaling pathways [17].

Irisin was discovered by Boström et al. in 2012 as a myokine stimulated by exercise, which is likely cleaved from the membrane protein FNDC5 (Fibronectin type III domain-containing protein 5) by an unknown protease [15]. Research has shown that exercise increases the expression of the peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC1α) in muscles. Activation of PGC1α stimulates the expression of the FNDC5 gene, leading to the synthesis of a protein of the same name. Irisin is mainly released from the fibronectin type III domain of this protein [15]. Therefore, it is suggested that circulating levels of irisin increase in individuals engaged in physical activity and decrease in sedentary individuals [15]. Following this study, various other studies examined the effect of exercise on the expression of FNDC5 or irisin. Kim et al. demonstrated that resistance training significantly increases circulating irisin levels in both rats and humans [18]. Similar results were found by Lee et al., where both resistance and endurance exercises were capable of inducing irisin secretion, although peak levels were observed following endurance exercises [19]. On the other forelimb, Amanat et al. showed that although 12 weeks of aerobic exercise, or aerobic combined with resistance exercise, increased serum irisin levels [20], only resistance exercise significantly increased irisin expression, a result corroborated by other studies [21, 22]. Findings regarding the effect of different types of exercise on FNDC5 gene expression are inconsistent. Therefore, the objective of the present study is to investigate the effects of resistance, endurance, and combined training on irisin levels and to identify the most effective exercise program for improving irisin levels. Additionally, the effects of these exercises on fast-twitch extensor muscles of the fingers and slow-twitch soleus muscles were compared.

MATERIALS AND METHODS

Animals and Study Protocol

This foundational research aimed to investigate the effects of three different types of exercise on FNDC5 gene expression in the muscles of rats. 40 healthy male Wistar rats, 8 weeks old, were selected. Initially, the rats were housed for two weeks under controlled conditions, with a temperature of 22±2°C, humidity of 50±5%, and a 12:12 hour light-dark cycle. This period allowed the rats to become familiar with their living environment, feeding conditions, and exercise protocols, minimizing stress and physiological changes.

The rats were housed in specialized cages (21 × 34 × 54 cm), made of polycarbonate, purchased from Razi Rad Industries. Wood chips were used on the cage floors to absorb urine and feces, ensuring the animals' comfort. To maintain a clean environment, the wood chips were replaced every two days, and the cages were washed weekly. Five rats were housed in each cage during the adaptation period. Due to the respiratory sensitivity of these rats to dust or ammonia from urine, a ventilation system was used in the animal room, running continuously.

All groups had free access to rodent-specific food pellets provided by Pars Animal Feed Company. The required amount of food was approximately 10 grams per 100 grams of body weight, and this amount was adjusted as the rats gained weight. In addition to food, the rats had access to sufficient water. A rat typically needs 10-12 milliliters of water per day per 100 grams of body weight. Water was supplied using 500-milliliter polycarbonate bottles, which were replaced and refilled daily.

During these two weeks, a motorized treadmill designed for animals (ST008, built by Tabriz University) was used to familiarize the rats with the exercise protocol. This treadmill featured five separate channels, allowing the control of parameters such as incline (positive and negative), speed, and time through a smart program. During this period, the treadmill was set to deliver an electric shock of 0.1 mV, with a 0% incline, a speed of 10-15 meters per minute, and an exercise duration of 5-10 minutes per day.

After the two-week familiarization period, the rats were randomly assigned to one of four groups based on their body weight: control, resistance, endurance, and combined exercise groups. Each group consisted of ten rats. The control group did not perform any exercise throughout the eight-week study period.

Exercise Protocol Implementation

Resistance Training Protocol

The resistance training program lasted 8 weeks, with 5 training days/week, similar to other groups. The resistance training method involved the rats performing three sets per day, with 6 repetitions in each set. The rest interval between sets was 3 minutes, and the rest interval between repetitions within a set was 45 seconds. All training sessions were conducted in the afternoon, between 3:00 and 6:00 PM. The warm-up and cool-down protocols were similar to those used by the other groups. For the resistance training, weights of varying percentages of the rats' body weight were attached to their tails, and they were trained on a surface inclined at 15 degrees. The weights started at 20% of the rats' body weight in the first week and increased by 10% each week, reaching 100% of their body weight by the 8th week. The detailed resistance training protocol is shown in Table 1.

Table 1. Eight-week resistance training schedule.

Resistance Training Protocol	The number of weeks of Exercise.					
Number of Sets	8	7	6	5	4	3
Number of Sets	3	3	3	3	3	3
Number of Repeats	6	6	6	6	6	6
Percentage ratio of weight to body weight	100	90	80	70	60	50

Endurance Training Protocol

The endurance training group also trained five days a week for eight weeks using a motorized animal treadmill. Since this study was conducted on animals and precise equipment for measuring exercise intensity was unavailable, treadmill speed and incline were used to control aerobic intensity. The endurance training protocol in the current study was based on the research by Nishio et al. (2001). These researchers, using available equipment, calculated the maximum oxygen consumption of the rats and determined the intensity of the exercise based on treadmill speed and incline for each training week. The test subjects in this study were matched to the Nishio study regarding strain, sex, age, and approximate weight. The treadmill incline was maintained at 15% throughout the training period. The speed started at 20 meters per minute in the first week and gradually increased to 30 meters per minute by the eighth week. Similarly, the duration of exercise began at 10 minutes per day in the first week and increased to 50 minutes per day by the eighth week. For the warm-up, the rats ran at a speed of 10 meters per minute on a flat treadmill for 5 minutes at the start of each session.

To reach the desired exercise intensity, the treadmill speed and incline were gradually increased over 5 minutes. At the end of the session, the incline was gradually reduced to 0 and the speed was slowly decreased to 10 m/min for a cool-down phase. The cool-down duration was approximately 5 minutes during the initial weeks and extended to about 10 minutes by the final weeks. All training sessions were conducted in the afternoon between 3:00 PM and 6:00 PM (Table 2).

Table 2. Eight week program of aerobic exercise.

Pppp	The number of weeks of Exercise.							
	1	2	3	4	5	6	7	8
Aerobic exercise protocol	10	10	20	30	30	40	40	50
Training duration (min/day)	20	20	24	24	26	26	30	30
Treadmil speed (m/min)	15	15	15	15	15	15	15	15

Concurrent Exercise Protocol

The concurrent exercise group performed a combination of resistance and endurance training for eight weeks, with five training days per week (Sunday, Monday, Tuesday, Thursday, and Friday). The rats in this group completed three endurance training sessions and three resistance training sessions each week. Two rest days per week were designated. On Friday mornings (9:00 AM to 11:00 AM), the rats underwent endurance training, and in the afternoon (4:00 PM to 6:00 PM), resistance training was conducted. On other days, the training sessions were held in the afternoon between 3:00 PM and 6:00 PM. The protocols for resistance and endurance training, as well as the warm-up and cool-down procedures, were the same as those used in the other groups.

FNDC5 Gene Expression Measurement

Tissue sampling from all animals was performed 48 hours after the final training session and following 12 hours of fasting. The rats were anesthetized via intraperitoneal injection of ketamine (90 milligrams per kilogram) and xylazine (10 milligrams per kilogram). They were then immediately euthanized and dissected by skilled professionals, and their body weight was measured using a digital scale. Based on the study's objectives, the desired tissues were extracted and placed in 1.5 or 2 microliter microtubes containing RNA Later and stored at -70°C. To preserve the tissue for RNA extraction, the following solution was used, prepared under a hood by combining the ingredients and adjusting the pH to 5 with sulfuric acid. The solution's final volume was adjusted to 1500 mL.

- Sodium citrate 1M (25 mL)
- Ammonium sulfate (700 g)
- Sterile distilled water (935 mL)
- EDTA 0.5 (40 mL)

In order to eliminate external RNase contamination, the disinfection solution below was prepared and used. Under the hood, the ingredients were combined and heated to mix thoroughly, then the final volume was brought to 500 mL using sterile distilled water. All equipment and surfaces used were disinfected with this solution to remove external RNase.

- H2O2 5% (71.42 mL)
- SDS 0.1% (5 mL)
- NaOH 0.05M (1 g)

For analyzing the expression of the desired genes or mRNA levels of specific proteins, the Real-Time PCR method was used. The tissue was placed on a slide and finely minced using scalpel and forceps. The minced tissue was then collected in the bottom of 2 microliter microtubes. Using a pipette, 1 mL of Plus-RNX kit (produced by Sina Gene Company) was added to each microtube. The tubes were vortexed for five seconds to ensure thorough mixing of the contents. The microtubes were incubated at room temperature for five minutes. Next, 200 microliters of chloroform were added to the samples, followed by 15 seconds of gentle manual shaking. The tubes were incubated on ice for five minutes and then centrifuged at 12000 rpm for 15 minutes at 4°C. After centrifugation, the microtubes were carefully removed without disturbing the contents, and 400 microliters of the upper aqueous phase were transferred to a new 1.5 microliter RNase-free microtube using a pipette, avoiding contact with the middle layer. The remaining liquid in the original microtube was discarded. Next, 400 microliters of isopropanol were added to the aqueous phase in the new microtube, gently mixed, and incubated on ice for 15 minutes. The tubes were then centrifuged again at 12000 rpm for 15 minutes at 4°C. After centrifugation, the supernatant was carefully discarded, leaving the RNA pellet. The pellet was then combined with 1 mL of 70% ethanol using a pipette and gently vortexed to break up the pellet and allow it to float. The tubes were centrifuged at 7500 rpm for 8 minutes at 4°C. After centrifugation, the supernatant was discarded, and the microtubes were inverted onto a gas sterilized with ethanol for 30 seconds, then placed in a rack to dry at room temperature. Once dry, 50 microliters of W.D. (Water DEPC) were added to each tube. The tubes were incubated at 60°C for 10 minutes in a water bath to dissolve the RNA pellet fully. At this stage, the RNA was ready for cDNA synthesis.

To measure and read the optical density (OD) using a spectrophotometer, first, 100 microliters of Water DEPC is placed in a cuvette as a blank. The cuvette is inserted into the spectrophotometer, and the blank button is pressed. Once the device is blanked and shows zero for OD, the blank cuvette is removed, and the sample cuvette is placed inside. For the sample, 99 microliters of Water DEPC and 1 microliter of the prepared RNA mixture are combined in a microtube and then transferred into the sample cuvette. The sample button is pressed to record the OD reading. For each OD reading, the spectrophotometer must be blanked, followed by the placement of the sample. The RNA 260/280 ratio must be greater than 1.6.

cDNA Preparation Using the Takara Viragen Kit

In order to prepare the PCR master mix for cDNA synthesis, all the following components are added to a microtube according to the kit's protocol, using a pipette. Then, 0.5 microliters of the target RNA is added to the master mix. The components are mixed on an ice bath.

- 5 X Prime Script buffer: 2 µL.
- Prime Script RT Enzyme Mix I: 0.5 µL.
- Oligo dt Primer: 0.5 µL.
- Random 6 mers: 0.5 µL.
- RNase Free dH_2O: 6.2 µL.

The required temperatures for carrying out the PCR reaction are as follows. At the end of the process, Mix Red was used to ensure cDNA synthesis.

Temperature	Time
37 ℃	15 min
85 ℃	5 seconds
4 ℃	

In order to perform PCR using the Mix Red from the Viragen company, the following materials were mixed in a microtube with cDNA according to the recommended protocol.

- Mix Red: 12.5 µL.
- Primer F: 1 µL.
- Primer R: 1 µL.
- cDNA: 1 µL.
- D.D.W: 9.5 µL.
- Total: 25 µL.

Then, one microliter of the synthesized cDNA was added to the master mix, and finally, PCR was performed in the machine according to the following temperature program. To confirm the desired cDNA synthesis, the product was loaded onto an agarose gel and then visualized under UV light to observe the specific band resulting from cDNA synthesis.

Temperature	Time
94 ℃	5 min
94 ℃	45 seconds
56 ℃	35 seconds
72 ℃	45 seconds
72 ℃	5 min

Preparation of 2% Agarose Gel and TAE Buffer

0.6 grams of agarose gel powder was weighed and placed into an Erlenmeyer flask. Then, 30 milliliters of 1X TAE buffer, measured with a graduated cylinder, was added to the powder in the flask. The flask was heated until the solution boiled, and the agarose powder dissolved in the buffer. This process (boiling stage) was repeated three times. Then, the flask was placed on a cloth to cool slightly. Two microliters of Safe DNA stain solution was added to the solution in the flask. The flask was swirled to mix the materials. The solution in the flask was gently poured into the prepared casting tray and combs. After the gel solidified in the tray for 20 to 30 minutes, the combs were carefully removed without damaging the gel. The tray was placed in an electrophoresis tank filled with TAE buffer. Then, 12.5 microliters of the desired cDNA was mixed with 2 microliters of loading buffer, and this mixture was loaded into the wells. The electric current was set to 100 volts. After loading the cDNA into the gel, it was transferred to a UV device to observe the desired band.

Preparation of TAE Buffer

All the following ingredients were combined, and the final volume was brought up to 1000 milliliters. This buffer is at a 50X concentration, and for use with the gel, 1X TAE buffer is required. Therefore, to dilute it, 20 milliliters of the above solution was measured with a graduated cylinder, transferred to a 1000-milliliter volumetric flask, and brought up to 1000 milliliters with distilled water.

- Acetic Acid: 57.1 ml.
- Na EDTA 0.5 M: 100 ml.
- Tris: 242 gr.

Primer Preparation

The primers were received in lyophilized vials and then diluted with TE buffer from Sina Gene at the ratio specified on the vial. Afterward, 180 microliters of TE buffer, 10 microliters of forward primer (F), and 10 microliters of reverse primer (R) were added to labeled tubes. The sequence, length, and type of primers designed for the FNDC5 gene are as follows.

- CCAAAAGTTCCAGGACTCAGG (F)21bp
- CCCGGTATCCCATTGTGACC (R)20bp

Primer Sequence for HPRT1 Gene:

- CTCATGGACTGATTATGGACAGGAC (F)25bp
- GCAGGTCAGCAAAGAACTTATAGCC (R)25bp

Primer Sequence for RPL13a Gene:

- GGATCCCTCCACCCTATGACA (F)21bp
- CTGGTACTTCCACCCGACCTC (R)21bp

After synthesizing the cDNA and primers, the gene sequences of interest were designed using Gene Script and Primer Design software, available on the NCBI website, and verified for accuracy with Blast and Oligo7 software. The PCR reaction was carried out using the Real Q Plus 2X Master Mix Green kit from Viragen, as outlined below.

Preparation of the Final Master Mix

Initially, 12.5 µL of SYBR Green Master Mix was pipetted into a microtube, followed by the addition of 0.5 µL of forward primer and 0.5 µL of reverse primer. Next, 10.5 µL of sterile distilled water was added to the mixture. Finally, 1 µL of the target cDNA was added to the solution. This combination, with a total volume of 24 µL, is sufficient for one reaction and one sample. For multiple reactions, the quantities of these components were multiplied by the number of samples. In this study, 40 samples of the target gene, 10 samples of the reference gene, and 2 NTC (No Template Control) samples, which included all components of the master mix except for cDNA, were prepared in 0.2 mL microtubes for each PCR cycle. All samples, including target and reference genes, were prepared in duplicate. These procedures were conducted under a hood in the dark and under sterile conditions. After preparation, the samples were placed in the machine, and the cycle count and temperature protocol were set as follows. After the experiment, the threshold line was adjusted in the Real-time PCR software for optimal results. The threshold line represents the level at which all samples begin to exponentially amplify. The cycle at which the threshold line intersects the PCR curve is called the Ct (Cycle threshold).

Cycle	Time	Temperature
1	15 min	95 ℃
40	15-30 seconds	95 ℃
	30 seconds	55-60 ℃
	30 seconds	72 ℃
	15 min	95 ℃

After the PCR reaction, the threshold cycles obtained from the samples of each group were collected in an Excel spreadsheet, where they were analyzed using the ΔΔCt and 2^(-ΔΔCt) formulas to compare the expression levels of target genes with the reference gene. The ΔΔCt method enhances sample compatibility without needing a standard curve when comparing gene expression levels relative to a reference control. For this method to be effective, the dynamic range of the target and reference genes must be similar and consistent.

- After completing the reactions, the average Ct for each sample was calculated. Then, the difference between the target and reference genes was calculated as ΔCt:

ΔCt = Ct (Target) – Ct (Reference)

- The difference between the control (natural samples) and treated samples was calculated as ΔΔCt:

ΔΔCt = ΔCt (Control) – ΔCt (Treated)

- These scales and data were converted to an absolute scale using the following formula:

Ratio = 2^(-ΔΔCt Target) / 2^(-ΔΔCt Reference)

Statistical Analysis

The Shapiro-Wilk test was used to assess the normality of the data distribution. A one-way ANOVA test was applied to compare the mean of the variable of interest across all groups. If significant differences were found, a post-hoc test was conducted to identify the exact differences between groups. The mean expression of FNDC5 between two muscles for each exercise was separately compared using a paired t-test to determine within-group differences. A significance level of $P < 0.05$ was used for all tests. All statistical analyses were conducted using SPSS software version 20.

RESULTS AND DISCUSSION

The results of the one-way ANOVA indicated that the different training programs (endurance, resistance, and combined) resulted in significantly different expression levels of the FNDC5 gene in both the forelimb muscle ($P < 0.001$) and the hindlimb muscle ($P < 0.001$) in comparison to each other and the control. These differences were also statistically significant (Table 3).

Table 3. Relative expression levels of FNDC5 gene in muscles of the forelimb and hindlimb of rats in different groups.

Tissues / Groups	Forelimb Muscle	Hindlimb Muscle	p-value*
Control Group	1	1	
Resistance Training	3.48±0.06	3.47±0.05	0.831
Endurance Training	2.48±0.04	2.49±0.05	0.636
p-Value**	<0.001	<0.001	

*The P-value obtained from the within-group comparison using the paired t-test.

** The P-value obtained from the between-group comparison using one-way ANOVA.

Additionally, a post-hoc test was conducted to compare all studied groups with each other. The results showed that FNDC5 gene expression in the forelimb and hindlimb muscles of all intervention groups was significantly higher than in the control group ($P < 0.001$). Furthermore, the intervention groups also displayed different levels of FNDC5 gene expression when compared to each other. Specifically, concurrent exercise had the greatest impact on FNDC5 gene expression compared to endurance and resistance training, and this difference was significant ($P < 0.001$). Additionally, FNDC5 gene expression in rats subjected to resistance training showed a significant increase compared to the endurance training group ($P < 0.001$) (Figures 1 and 2).

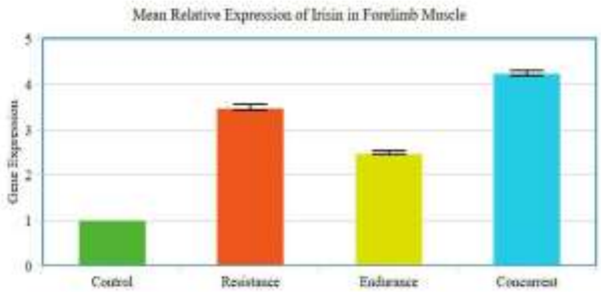

Figure 1. Comparison of Relative Expression Levels of the FNDC5 Gene in the forelimb Muscle Across Different Groups.

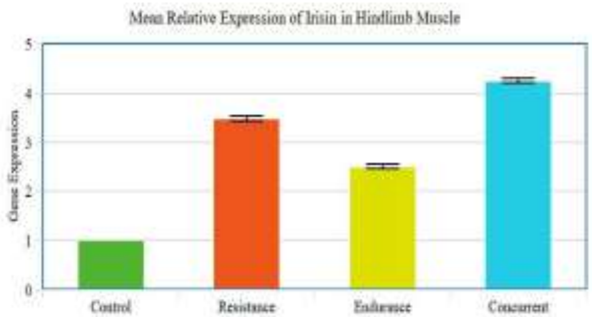

Figure 2. Comparison of Relative Expression Levels of the FNDC5 Gene in the Hindlimb Muscle Across Different Groups.

In order to compare the differences in FNDC5 gene expression between the forelimb and hindlimb muscles within each group, a paired t-test was used, with results displayed in Table 3. The analysis indicated no significant differences in FNDC5 gene expression between the forelimb and hindlimb muscles in any of the training groups: resistance ($P = 0.831$), endurance ($P = 0.636$), and concurrent ($P = 0.920$).

The results of present study showed an increase in FNDC5 gene expression following eight weeks of endurance, resistance, and concurrent training compared to the control group. Among the training groups, concurrent exercise had the greatest impact on FNDC5 gene expression, followed by resistance and endurance training, respectively. No significant differences were observed when comparing FNDC5 gene expression between the forelimb and hindlimb muscles.

Athough irisin is often recognized as an exercise-induced myokine, the results from various studies in this area are heterogeneous. For instance, Timmons et al. analyzed the effects of endurance and resistance exercises on young and older groups and found that only older individuals reported significant differences in FNDC5 gene expression with endurance training [23]. This research suggested that FNDC5 might not be systematically affected by exercise. In another study by Hecksteden et al., contrasting the present study, they found that neither aerobic nor resistance exercises had a significant effect on serum irisin levels [24]. However, it's important to note that Hecksteden's study measured serum irisin levels, while our study examined FNDC5 gene expression at the tissue level. Another study that investigated both serum levels and gene expression of irisin after exercise found that an increase in FNDC5 mRNA does not necessarily result in higher serum irisin levels [25]. Conversely, other studies have shown that FNDC5 gene expression increases in response to exercise, which subsequently leads to elevated circulating irisin levels [15, 26]. For example, research by Boström et al. demonstrated that PGC1-α gene expression in muscle leads to increased expression of the FNDC5 membrane protein. This process results in the release of irisin, a myokine, which slightly elevates blood irisin levels, enhancing energy expenditure in rats without affecting their movement or food intake. As a result of increased irisin, obesity and glucose homeostasis improved. Therefore, irisin appears to be a key mediator in metabolic diseases and other disorders that benefit from exercise [15]. In another study by Abdi et al., the effect of concurrent exercise on irisin and FNDC5 gene expression in abdominal fat tissue in diabetic rats was evaluated. The results indicated that concurrent exercise significantly increased FNDC5 gene expression, though the rise in irisin levels, while observed, was not statistically significant [27]. It seems that health status may also influence irisin secretion and the release of this myokine from the FNDC5 membrane protein.

Numerous clinical trials have reported the effects of exercise on irisin secretion levels. In a study by Kim et al., the effects of aerobic and resistance training on circulating irisin levels and their relationship with changes in body composition were examined in overweight/obese adults. In this study, 28 overweight/obese adults were compared before and after an 8-week exercise program (60 minutes per day, 5 days a week). Participants showed significant improvements in body composition parameters and exercise capacities, such as maximum oxygen uptake and muscle strength, in both aerobic and resistance training. Interestingly, circulating irisin levels increased significantly in the resistance training group compared to the control, but this increase was not significant in the aerobic group. Additionally, there was a positive correlation between changes in circulating irisin and muscle mass, and a negative correlation between changes in circulating irisin and fat mass. The findings suggested that resistance training might be an effective exercise for overweight/obese individuals, given the positive body composition changes along with increased irisin levels [28]. The duration and frequency of exercise in that study were similar to those in the present study. Their findings also align with our research, which reported the greater effectiveness of resistance over endurance exercise. However, another study comparing the effects of high-intensity endurance exercise with resistance exercise found that after ten weeks, irisin levels were higher in rats undergoing high-intensity endurance exercise compared to the control and resistance groups [29]. Therefore, it appears that not only the type of exercise but also the intensity can influence the secretion of myokines. This claim was also reported in another study by Enteshari et al., which examined the effects of eight weeks of combined exercise at two intensities—moderate and high—on irisin levels compared to a control group in women with type 2 diabetes. The results indicated that both exercise groups had higher irisin levels compared to the control at the end of the study, with the increase being significantly higher in the high-intensity group [30]. Similarly, in a study by Ghaderi et al., it was shown that endurance exercise, regardless of intensity, can increase FNDC5 gene expression in skeletal muscles; however, exercise intensity plays a role in breaking down FNDC5 protein and releasing irisin into the bloodstream. High-intensity exercise had a more pronounced effect on serum levels of this myokine [31].

In another study aimed at examining the acute effects of exercise on serum irisin, participants were divided into three groups—resistance, endurance, and concurrent—and each performed one hour of exercise specific to their group. The results showed that irisin levels in the resistance group increased significantly more than in the endurance and concurrent groups. Additionally, the lowest increase was observed in the concurrent group, which contrasts with the current study's findings [22]. This suggests that exercise duration may also be a significant factor in determining irisin expression levels. In another study by Nygaard et al., the effects of a single session of high-intensity endurance exercise and a single session of heavy resistance training were compared with resting conditions. Results indicated that serum irisin levels peaked immediately after exercise in the endurance group and 1 hour after exercise in the resistance group. Although this increase was temporary, levels gradually returned to baseline. However, FNDC5 gene expression measured four hours after exercise was not significantly affected in either exercise group, despite both protocols leading to a significant increase in PGC-1α gene expression, which plays a role in regulating FNDC5 transcription [32]. Therefore, it seems that the increase in irisin following short-term exercise may be due to the breakdown of pre-existing FNDC5 protein, and short-term exercise may not have a significant effect on gene expression.

A positive point of this study is its innovation, as no prior research has compared the effects of different exercise modes on FNDC5 gene expression in muscles. Since physical exercise primarily impacts muscle function, the effect of exercise on muscle metabolites is particularly important. However, this study has limitations. Possible limitations include the lack of serum irisin measurement, the absence of human samples, the lack of biochemical factors related to irisin function, the absence of body composition and metabolism measurements, and the lack of control over the rats' activity levels in their cages.

Based on the findings of the present study, all three types of exercise significantly increased FNDC5 gene expression compared to the control group. However, the degree of effect varied across the different types of exercise, with concurrent exercise, resistance, and endurance showing the highest impact on FNDC5 gene expression, respectively. Nonetheless, studies on the effect of exercise on the myokine irisin remain highly inconsistent, and it appears that various factors beyond exercise type, such as duration, intensity, body composition, and health status, may influence irisin secretion. Therefore, further research is recommended to better understand the factors affecting irisin-related gene expression and the health outcomes of irisin level changes.

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