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Original Article

Effect of the Aerobic and Resistance Training on Follistatin-Like 1 and Leukemia Inhibitory Factor Muscle Gene Expression in Rats Fed With a High-Fat Diet

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Abstract

Background: Follistatin-like 1 (FSTL-1) and leukemia inhibitory factor (LIF) are two myokines that are affected by overweight and have inflammatory and damaging effects. Considering that exercise reduces excess weight, this study aimed to evaluate the effect of aerobic and resistance training on FSTL-1 and LIF muscle gene expression in rats fed with a high-fat diet.

Materials and Methods: In this experimental study, 32 rats were randomly divided into healthy control, obese control, obese + aerobic exercise, and obese + resistance exercise groups. The training was performed for 4 weeks at aerobic moderate intensity (50-65% $VO2_{max}$). For resistance training, rats were also trained to climb the ladder (height 110 cm, slope 80%, and the distance between the bars of the ladder 2 cm), which is based on the determination of one repetition maximum. A high-fat diet was prepared with 40% fat, 13% protein, and 47% carbohydrates and continued until the rats reached the obesity range. The tissue sample was taken from the gluteus muscle.

Results: The expression of FSTL-1 and LIF in the obese control group increased significantly compared to the healthy control group ($P=0.044$ and $P=0.039$, respectively). The expression of FSTL-1 and LIF in the resistance training group significantly decreased in comparison to the obese control group ($P=0.049$ and $P=0.046$, respectively). There was no significant difference between the aerobic exercise group and the obese control group ($P=0.053$ and $P=0.059$, respectively). However, a significant difference was observed between aerobic and resistance training groups in terms of FSTL-1 ($P=0.042$).

Conclusion: Resistance exercise seems to have a greater and better effect on FSTL-1 and LIF in the muscles of obese samples compared to aerobic exercise.

Keywords: Exercise training, Myokines, Obesity

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Introduction

Myokine is a cytokine released by skeletal muscle cells (muscle fibers) in response to muscle contractions (1). Muscle cells have paracrine and/or endocrine and autocrine effects. Their systemic effects occur at picomolar concentrations. Myokine receptors are found on fat, muscle, pancreas, liver, bone, immune system, heart, and brain cells (1). In this regard, Licursi et al reported that the reduction of the anorectic leukemia inhibitory factor (LIF) in the brainstem caused by a high-fat diet may provide the right conditions for the overconsumption of a high-fat diet (2). This process may be mediated, at least in part, by the nucleus of the tractus solitaries (2). Johnson et al found that LIF reduced body fat mass in ovariectomized rodent rats. LIF is one of the discovered myokines (3). It is now known that LIF has a wide range of actions, including acting as a stimulator for platelet formation, hematopoietic cell proliferation, bone formation, neurogenesis and survival, muscle satellite cell proliferation, and acute phase hepatic cell production (4).

On the other hand, Follistatin-like 1 (FSTL-1) is a secreted extracellular glycoprotein that has been identified as a new pro-inflammatory cytokine. FSTL-1 was originally cloned from an osteoblast cell line as a *transforming growth factor-β* inducible gene (5). Based on sequence homology, FSTL-1 belongs to the BM/SPARC/osteonectin family, which contains two calcium-binding and follistatin-like extracellular domains (5). However, the calcium-binding domains of FSTL-1 are considered non-functional (5), suggesting that FSTL-1 may have distinct functional properties. With a broad expression pattern (e.g., lung, heart, brain, and urinary tract), FSTL-1 has demonstrated diverse and cell-specific functions, including the regulation of cell proliferation, apoptosis, differentiation, and migration (5). Consequently, FSTL-1 is involved in several biological processes

such as angiogenesis, tumorigenesis, and embryonic development (5). In this regard, Horak et al demonstrated that it is biologically possible that overweight and mild obesity are associated with increased levels of FSTL-1, mainly due to its pro-inflammatory activity during this chronic inflammatory state with a lower grade and higher number. Preadipocytes preferentially express FSTL-1 (6). On the other hand, morbid and extreme obesity is potentially associated with decreased FSTL-1 levels due to the persistent loss of adipogenesis and increased number of mature adipocytes, increased cellular senescence, increased demand for the decreased anti-apoptotic activity of FSTL-1, and possibly also, it is related to epigenetic gene silencing by methylation. Furthermore, these changes in FSTL-1 protein levels were not affected by gender because FSTL-1 was equally expressed in both men and women in both obese and non-obese groups. Fan et al reported that FSTL-1 impairs insulin signaling in 3T3-L1 adipocytes, as manifested by the impaired phosphorylation of protein kinase Band insulin receptor substrate-1 in response to insulin stimulation (5). It was also reported that FSTL-1 is a potential mediator of inflammation and insulin resistance in obesity.

Although overweight and mild obesity are associated with increased levels of FSTL-1, studies investigating the relationship between exercise and FSTL-1 have mostly focused on acute rather than long-term effects. All types of exercise, including resistance (7), endurance, and high-intensity interval training, increase plasma/serum FSTL-1 (8, 9). The increase in the FST concentration ranges from about 5% to 500%. The strongest response was observed in younger subjects who exercised at a higher intensity. These characteristics may be the limiting factors for increasing the serum/plasma concentration of FSTL-1 L-1 in other studies. FSTL-1 concentrations peak 3-4 hours after exercise and then represent a decline. However, in some studies, high concentrations were observed for 72 hours (10). The effects of regular exercise were investigated in exercise-based studies from 2 weeks to 6 months. In serum/plasma FSTL-1 studies, regular physical activity increases its baseline concentration regardless of the duration of exercise and the type of the performed activity. However, it should be emphasized that participants in these studies were mostly middle-aged and elderly. It seems that there is no clear opinion about the effect of long-term exercise, especially in the obese sample, on FSTL-1. Regarding the effect of exercise on LIF, no exact results are available; however, Broholm and Pedersen reported that during and after exercise, skeletal muscles synthesize and release a number of myokines that exert their effects either systemically or locally in the muscle (4). Given the above-mentioned explanations, this study sought to investigate the effect of the type of exercise (aerobic-resistance exercise) on the expression of LIF and FSTL-1 genes in rats fed with fatty food.

Materials and Methods

This was experimental and fundamental research. The statistical population of this study included all adult male Wistar rats (12 weeks old, with an average weight of 180-200 g) and were included in 4 groups (8 rats in each group). The rats were kept under standard environmental and temperature conditions with 12 hours of light and 12 hours of darkness in standard-sized cages. Five rats were kept in each cage.

One week before the start of the training protocol, the animals were kept at the project implementation site in order to adapt to the new environment, and during the study period, all the animals were kept under standard laboratory conditions in clear polycarbonate cages with autoclave dimensions (15×42×5), temperature (20-22°C), humidity (55%), and free access to water (a 300 mL transparent and graduated bottle with autoclave capability and a 1 cm stainless steel cap without thread) with a 12-hour dark/light cycle was maintained. All the principles of working on laboratory animals approved by the Ministry of Health of the Islamic Republic of Iran were observed in this study (11).

Overall, 32 rats were randomly divided into four groups, including healthy control, obese control, obese + aerobic exercise, and obese + resistance exercise. Aerobic and resistance training was performed for 4 weeks and 3 days a week.

To create an obesity model, a high-fat diet was prepared with 40% fat, 13% protein, and 47% carbohydrates and continued until the rats reached the obesity range (12). The rats were fed with the prepared diet for 9 weeks, and the training protocol was started after they reached the desired weight. The standard food of laboratory animals was used for healthy control group rats.

Aerobic Exercise Program

An aerobic exercise program was implemented for 4 weeks with moderate intensity. To adapt the rats to the main training program, a week of adaptation training was performed at a speed of 9 meters per minute for 20 minutes. Regarding the main training, the training intensity reached 50% $VO2_{max}$ and 65% $VO2_{max}$ in the first and last weeks, respectively. Accordingly, the duration of training was determined to be 20 minutes, and the training intensity reached 16 and 26 meters per minute in the first and last weeks, respectively. In addition, the rats were warmed up for 5 minutes at a speed of 7 m/min to start the training and cooled down for 5 minutes at a speed of 5 m/min after the main training (13).

Resistance Training Protocol

The rats were trained to climb a ladder (height 110 cm, incline 80%, and a distance between rungs 2 cm), which is based on the determination of a one-repetition maximum

(1RM). The weight of the mice was measured after one week of adaptation. Then, a weight equal to 50% of their body weight was attached to the end of their tail. After one successful ascent, 30 g were added to the initial weight (50 + 30% g). The last weight the animal could lift was considered 1RM. The first training session started with 50% and 140 seconds of rest between each set and continued with 75%, 90%, and 100% of 1RM. If the animal could lift 100% 1RM, 30 g was added to the weight, and this process continued until the animal could not reach 1RM. The last successfully lifted weight was considered the rat's 1RM. On the following days, the training was started with the highest weight of the previous day calculated as 1RM (8).

Tissue Method

In this method, 48 hours after the last intervention, all rats fasted for 8-10 hours, and weight was taken before tissue removal. The anesthetic drug was a combination of 10% ketamine and 2% xylazine, and the selected dose for ketamine was 100 mg/kg and xylazine 10 mg/kg. The muscle tissue sample was taken from the gluteus muscle.

Polymerase Chain Reaction Method

The quantitative polymerase chain reaction (qPCR) method was used to investigate the expression of genes in the gluteus muscle tissue. In this study, the GAPDH reference gene was employed as a control gene, and the expression of other genes was compared with it. To perform this technique, first, the primers were designed, and then total RNA was extracted from the tissues and converted into cDNA. Next, the cDNA was amplified by the PCR and analyzed for the expression of the mentioned genes (Table 1).

Subsequently, RNA was extracted by a manual method using Trizol material prepared from Kiazist Company (Iran) and according to the existing standard protocol for the Trizol method.

Moreover, cDNAs were synthesized using the Parstous cDNA synthesis kit (Parstous, Mashhad, Iran; Catalog number: A101161). Further, the primers were designed with Gene Runner, version 6.5.

In addition, the PCR method was performed using Korea's BioFact kit: 2X Real-Time PCR Master Mix, including SYBR Green, High ROX; Cat No. DQ385-40h). The expression ratio of the studied genes in this study was evaluated by the comparative method of the threshold cycle.

Primer sequences and characteristics are provided in Table 1. The amplification was conducted at 95˚C for 15 minutes, followed by 35 cycles of 40 seconds at 95˚C, 25 seconds at 62˚C, and 15 seconds at 72˚C. A melting curve from 55˚C to 100˚C was recorded to detect potential unintended products.

Statistical Analysis

In this research, the Shapiro-Wilk test was used to check the normality of data distribution. When the data distribution was normal, a one-way analysis of the variance (ANOVA) test was applied to check the difference between groups, and Tukey's post hoc test was utilized to determine the place of the difference between groups. All analyses were performed using SPSS software (version 22) at the level of $P \leq 0.05$.

Results

The results of this study (Figure 1) demonstrated a significant difference between the groups in terms of body weight ($P = 0.031$). The amount of weight in the aerobic training group was significantly reduced compared to the obesity control ($P = 0.041$). Conversely, no significant change was observed in the resistance training group ($P = 0.054$).

According to the ANOVA test results, a significant difference was found between groups in terms of FST L-1 expression ($P = 0.049$). Based on the post hoc test, the expression of FST L-1 in the obese control group increased significantly compared to the healthy control group ($P = 0.044$), while that of the resistance training group significantly decreased in comparison to the obese control group ($P = 0.049$). However, despite the increase in FST L-1 in the aerobic exercise group compared to the obese control group, there was no significant difference between these two groups ($P = 0.053$). Contrarily, a significant difference was detected between aerobic and

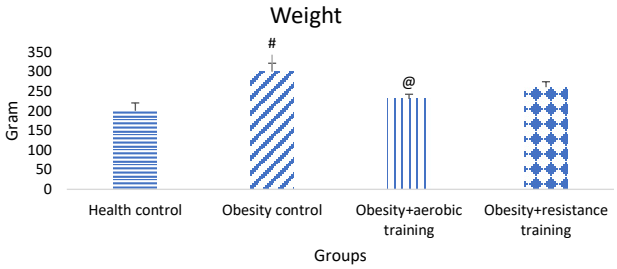

Figure 1. Body Weight in All Groups *Note*. # Significant compared to health control; @ Significant in comparison to obese control.

Table 1. Primers

Gene	Forward Primer (5´–3´)	T_m	GC%	Reverse Primer (5´–3´)	T_m	GC%	Product Size (bp)
GAPDH	5-AACCCATCACCATCTTCCAG-3	60.4	55	5-CCAGTAGACTCCACGACATAC-3	61.2	56	183
FSTL1	5-AGAACGCTGACTGGAAACTC-3	59.4	55	5-AGGAACAGACACAGCGATTG-3	59.6	59.6	141
LIF	5-AAGTTGGTCGAGCTGTATCG-3	60.1	55	5-GTCGCATTGAGTTTGATCTGG-3	59.2	532	235

Note. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; FSTL1: Follistatin-like 1; LIF: Leukemia inhibitory factor; G: guanine; C: cytosine.

resistance training groups ($P=0.042$, Figure 2).

Additionally, ANOVA test results (Figure 3) demonstrated a significant difference in LIF expression between groups ($P=0.041$). Based on the post hoc test results, the expression of LIF in the obese control group increased significantly in comparison to the healthy control group ($P=0.039$). Conversely, the expression of LIF in the resistance training group significantly decreased compared to the obese control group ($P=0.046$). However, despite the reduction of LIF in the aerobic training group when compared with the obese control group, there was no significant difference between these two groups ($P=0.059$). Finally, a significant difference was found between aerobic exercise and resistance exercise groups ($P=0.055$).

Discussion

The research results revealed that the expression of FSTL-1 was significantly decreased in the resistance training group. However, aerobic exercise had no significant effect on the expression of FSTL-1. Consistent with our findings, some studies showed that serum FSTL-1 levels were increased in overweight/obese patients compared to healthy adults, and adipose FSTL-1 expression levels were increased in db/db rats in comparison to wild-type rats (14). Conversely, in a study, the expression level of adipose FSTL-1 was not changed in healthy-sedentary, obese-sedentary, and obese exercising rats (15).

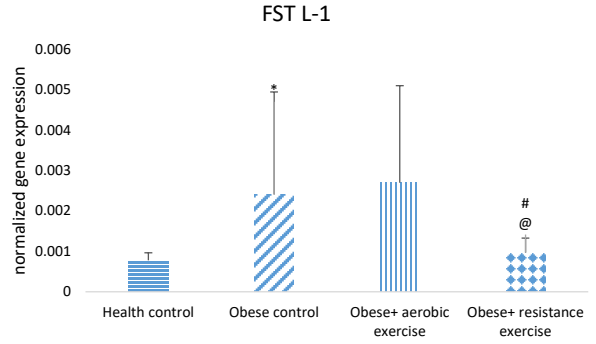

Figure 2. FSTL-1 Expression in All Groups
Note. Follistatin-like 1; * Significant compared to healthy control; # Significant compared to obese control; @ Significant compared to obese + aerobic exercise.

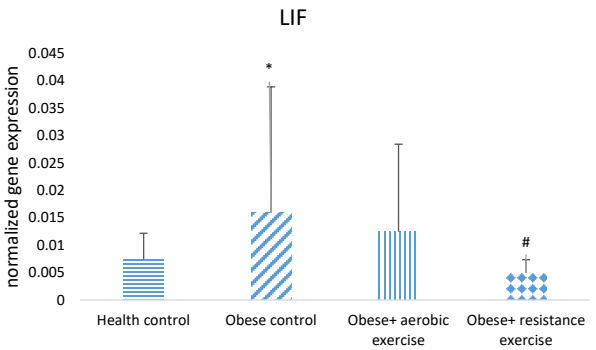

Figure 3. LIF Expression in All Groups. *Note*. LIF: Leukemia inhibitory factor; *Significant compared to healthy control; # Significant in comparison to obese control.

Inoue et al reported that the FSTL-1 levels of slow-twitch fiber-rich plantar soles increased under the influence of aerobic exercise and were positively correlated with the serum levels of FSTL-1 (15). In contrast, fast-twitch fiber-rich tibialis anterior FSTL-1 levels did not change and did not correlate with serum FSTL-1 levels. These results indicate that the response of FSTL-1 expression to aerobic training is different between slow-twitch and fast-twitch fibers. Therefore, the expression of FSTL-1 due to aerobic training in slow-twitch fibers may help increase the level of circulating FSTL-1 (15). Another study using muscle-specific FSTL1-deficient and muscle-specific FSTL-1 overexpressing rats confirmed that skeletal muscle is one of the main secretory sources of FSTL-1 (16).

Likewise, Tolouei Azar et al investigated the effect of six weeks of aerobic training on FSTL-1, *vascular* endothelial growth factor, brain-derived neurotrophic factor, and vascular changes in healthy male rats (17). The results represented that six weeks of endurance training significantly increased FSTL-1 levels. Interval training (treadmill running, alternating between 7 minutes at 25 m/min and 3 minutes at 15 m/min) induced myocardial FSTL-1 expression and reduced cardiac dysfunction in a rat model of myocardial infarction (18). However, in the study by Xi et al, treadmill exercise at a speed of 25 m/min did not affect myocardial FSTL-1 levels (18). On the other hand, Xi et al reported that resistance exercise increases the regulation of skeletal muscle FSTL-1 and thus improves cardiac angiogenesis in rats with myocardial infarction (19).

It seems that the reason for the difference in our results with the findings of other researchers can be related to the examination of FSTL-1 in the serum or its examination in the tissue. Considering that FSTL-1 can be released by various tissues, the plasma levels of FSTL-1 are increased by resistance exercise. However, its tissue levels decrease, especially in the muscles of obese samples, as mentioned in our research. In addition, the obesity and overweight of the samples affect the results of the research.

Furthermore, the results of the research test revealed that the expression of LIF in the resistance training group was significantly decreased in comparison to the obese control group. However, despite the reduction of LIF in the aerobic training group compared to the obese control group, there was no significant difference between these two groups. However, there was a significant difference between aerobic and resistance training groups.

In this regard, the results of some studies demonstrated that LIF levels in circulation are related to the severity of liver steatosis. Patients with ballooning, fibrosis, lobular inflammation, and abnormal increase of liver damage markers (i.e., alanine transaminase and aspartate aminotransferase) also had higher levels of serum LIF

than control patients (20). In this respect, So concluded that 12 weeks of aerobic and resistance training decreased the level of LIF protein in the hind limb muscle in rats, and there was a negative correlation between the level of LIF protein in the soleus muscle and the fat-free mass of the lower leg (20).

In another study, eight men cycled for 3 hours at 60% $VO2_{max}$, and muscle samples were taken before exercise and up to 48 hours after exercise. Muscle LIF mRNA expression increased up to 4-fold immediately after the cessation of the exercise and gradually decreased during the post-exercise period (21). The findings of this study showed that aerobic exercise and resistance muscle contractions regulate muscle LIF mRNA expression in humans. Considering that resistance training causes more muscle involvement, the decrease in LIF in our research in the resistance training group may be the reason.

According to the results of this research, a fatty food diet increased the expression of LIF and FSTL-1 in the muscle tissue of rats. Although resistance training decreased FSTL-1 and LIF, aerobic training had no significant effect. In general, resistance exercise seems to have a greater and better effect on myokines in the muscles of obese samples compared to aerobic exercise. However, more research is needed in this regard. With certainty, we cannot state a clear mechanism for the greater effect of resistance training. However, some studies reported that the DIP2A-Smad2/3 signaling pathway is effective in this field (22).

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Authors' Contribution

Conceptualization: Mostafa Babaeinejad.
Data curation: Mostafa Babaeinejad, Hasan Matinhomaee.
Investigation: Mostafa Babaeinejad, Hasan Matinhomaee.
Methodology: Mostafa Babaeinejad.
Project administration: Mostafa Babaeinejad, Hasan Matinhomaee.
Supervision: Hasan Mateen Homaie, Hoseyn Fatolahi.
Writing–original draft: Mostafa Babaeinejad.
Writing–review & editing: Hasan Matinhomaee, Hoseyn Fatolahi.

Competing Interests

The authors of this paper reported no conflict of interest.

Ethical Approval

This study was approved by the Ethics Committee of Islamic Azad University, Central Tehran Branch (With the code IR.SSRI.REC.1401.1607).

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