Effect of aerobic and resistance training on secreted protein acidic rich in cysteine, decorin, and myonectin muscle genes expression in rats fed with a high-fat diet

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Abstract

Background and aims: Fatty diet can affect the expression of myokines. Thus, the aim of this study was to evaluate the effect of aerobic and resistance training secreted protein acidic rich in cysteine (SPARC), decorin (DCN), and myonectin muscle gene expression in rats fed with a high-fat diet.

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 program was implemented for 4 weeks at aerobic moderate intensity (50% VO2max in the first week and 65% VO2max in the last week). For resistance training, the animals 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 (1RM). Obese rats received a dose of 0.5 mL/100 g of body weight orally by gavage for 4 weeks and 5 days a week.

Results: There was no significant difference between the groups in terms of DCN expressions (P = 0.702). However, the expression of SPARC (P = 0.039) and myonectin in the obese control group was significantly increased compared to the healthy control (P = 0.038). The expression of SPARC and myonectin in the aerobic training group (P = 0.038, P = 0.042) and the resistance training group (P = 0.048, P = 0.049) was significantly decreased in comparison to obesity control. Resistance training caused a greater decrease in myonectin compared to aerobic training (P = 0.049).

Conclusion: The findings revealed that aerobic and resistance exercises are effective in reducing SPARC and myonectin expression in the skeletal muscle of rats fed with a high-fat diet.

Keywords: SPARC, Decorin, Myonectin, Obesity, Exercise training

Introduction

Being overweight and having a high-fat diet can affect myokine levels (1). Myokine is a small product (~5-20 kDa) and includes proteoglycan peptides that are released by skeletal muscle cells (muscle fibers) in response to muscle contractions (2). The receptors of myokines are found in the cells of the muscle, heart, immune cells, and brain (2). In this regard, secreted protein acidic rich in cysteine (SPARC) is a cytokoregulatory macromolecule that does not contribute to matrix structure but regulates cell-matrix interactions (3). Immunostaining with the fibrosis marker picrosirius red has shown more fibrosis in the subcutaneous tissue of people with severe obesity than in the lean control group. The increased fibril-forming structure increases stiffness and compromises differentiating cells (3). SPARC secretion in obese mice increases with weight gain, while it decreases with diet-induced weight loss (4). Increased SPARC secretion increases adipose tissue fibrosis and fat deposition in various organs and contributes to insulin resistance (5).

On the other hand, the small leucine-rich proteoglycan decorin (DCN) is a component of the extracellular matrix (ECM) in many tissues (6). Experiments with dissected adipose tissue show that DCN expression is the highest in the non-fatty stromal vascular fraction that contains, for example, fat precursors (7). Previous studies on obese individuals with glucose intolerance have demonstrated an increase in DCN mRNA expression (8). DCN may influence adipose tissue metabolism and expansion (9). In addition, DCN interacts with several molecules present in the ECM (6). These interactions may be involved in regulating the metabolism and activity of these growth factors in their receptors in addition to stabilizing the ECM (6).

On the other hand, myonectin expression is stimulated by two main factors, namely, exercise and diet (10). Researchers represented a decrease in myonectin levels in rats fed with a high-fat diet, which was caused by a decrease in mRNA levels in skeletal muscles (11). Such results indicated that the decrease in circulating myonectin
levels can help reduce the absorption of free fatty acids in the adipose tissue and thus increase the circulating level and ectopic accumulation in other tissues. Patterson et al reported increased circulating myonectin in obese Zucker rats (12).

It seems that exercise can be effective in regulating myokines. However, the type of exercise is highly important. Few studies have investigated the potential role of DCN in exercise. Kanzleiter et al found that DCN plasma concentration is increased in humans who perform acute resistance training (13). Knuiman et al reported that DCN concentration was not significantly different between baseline and after endurance and resistance training (14). Son et al concluded that resistance training led to the suppression of SPARC expression in the soleus and confirmed the effect of pre-training on atrophied muscles. Therefore, SPARC may play an important role in muscle homeostasis (15). Pourranjbar et al investigated the effect of aerobic exercise on myonectin serum levels and insulin resistance in obese and overweight women and found that the myonectin serum level increased significantly in the experimental group (16). Peterson et al showed that exercise did not increase the expression of FNDC5 or myonectin genes (12). In general, the effect of exercise on the expressed myokines has not been clearly defined yet. In addition, there is no consensus about which type of exercise can lead to better effects. We have not yet achieved clear results, especially in obese or high-fat diet samples. Therefore, this research aimed to evaluate the effect of aerobic exercise and resistance training on the expression of SPARC and DCN genes in rats fed with fatty diets.

Methods
The statistical population of this experimental and fundamental research included all adult male Wistar rats, 12 weeks old, with an average weight of 180-200 g, which were used 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 each including 5 animals.

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. During the study period, all the animals were kept under standard laboratory conditions in clear polycarbonate cages with autoclavable dimensions (15 × 42 × 5). Temperature (20-22 °C), and humidity (55%). Further, they had 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. All principles of working on laboratory animals approved by the Ministry of Health of the Islamic Republic of Iran were observed in this study.

Overall, 32 rats were randomly assigned to healthy control, obese control, obese+aerobic exercise, and obese+resistance exercise groups. To create an obesity model, all rats received a dose of 0.5 mL/100 g of body weight orally by gavage for 4 weeks and 5 days a week (17).

Aerobic exercise program
The training program was performed for 4 weeks at aerobic moderate intensity. Based on studies, the training intensity reached 50% VO_{2max} in the first week and 65% VO_{2max} in the last week. To acclimatize the rats, before starting the main training program, one week of acclimatization training is performed at a speed of 9 m/min for 20 minutes. According to previous studies, the duration of training was fixed at 20 minutes, and the intensity of training reached 16 m/minute on the first day and 26 m/min on the last day. To start training, warm-up and cool-down exercises were performed for 5 minutes at a speed of 7 m/min and 5 minutes at a speed of 5 m/minute after the main training, respectively (18).

Resistance training protocol
Rats were 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 (1RM). After one week of adaptation, the weight of the mice will be taken, then a weight of 50% of their body weight will be attached to the end of their tail. After one successful climb, 30 g will be added to the initial weight (50% + 30 g). The last weight that the animal could lift was considered as 1RM. The first training session started with 50% and with a rest interval of 140 seconds between each set and continued with 75%, 90%, and 100% of 1RM. If the animal could raise 100% of 1RM, 30 g were added to the weight (100% + 30 g), and this process continued until the animal was unable to reach 1RM. The last weight that was successfully lifted was considered the 1RM of that mouse. On the following days, the training with the highest weight of the previous day was started as the calculated 1RM (18).

Tissue method
In this method, 48 hours after the last intervention, all rats were 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 (PCR) method was used to investigate the expression of genes in the heart tissue. In this study, the reference gene glyceraldehyde-3-phosphate dehydrogenase was employed as a control gene, and the expression of other genes was compared with it. To perform this technique, first, primer design was performed, 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).

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

Moreover, cDNAs were synthesized using the Parstous cDNA synthesis kit (Parstous, Mashhad, Iran; Cat No.: A101161). Further, 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). Table 1 presents the sequence of primers used in the research, along with the control gene.

### Statistical method

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

### Results

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

Based on ANOVA results, there was no significant difference between the groups in terms of DCN expression ($P = 0.702$). Thus, despite the increase of DCN in the obese control group compared to the healthy control, this increase was not significant ($P = 0.891$). In addition, DCN expression decreased in the aerobic ($P = 0.661$) and resistance ($P = 0.83$) training groups in comparison to the diabetes control group, but the changes were not significant. No significant difference was found between the two groups of aerobic exercise and resistance exercise in terms of DCN expression ($P = 0.89$, Figure 2).

Furthermore, ANOVA results revealed that there is a significant difference between the groups in terms of SPARC expression ($P = 0.04$). Based on the results of the post-hoc-up test, the expression of SPARC in the obese control group was significantly increased compared to the healthy control ($P = 0.039$). However, the expression of SPARC in the aerobic ($P = 0.038$) and resistance ($P = 0.048$) training group was significantly decreased in comparison to obesity control. No significant difference was observed between aerobic and resistance training groups in terms of SPARC expression ($P = 0.77$, Figure 3).

ANOVA test results showed a significant difference in the expression of myonectin between the groups ($P = 0.044$). According to the post hoc test, the expression of myonectin in the obese control group was significantly increased compared to the healthy control ($P = 0.038$). Conversely, myonectin expression was significantly decreased in the aerobic and resistance training groups when compared to the obese control group ($P = 0.042$). There was a significant difference between the aerobic and resistance training groups ($P = 0.049$, Figure 4).

### Discussion

The results of this research demonstrated that SPARC increased significantly due to a fatty diet, and aerobic exercise and resistance exercise decreased its expression. Although the decrease in SPARC expression was higher in the aerobic exercise group, there was no significant difference between the effect of resistance and aerobic exercise. Other studies reported that feeding mice with a high-fat diet for 12 weeks increased SPARC expression in adipose tissues at both mRNA and protein levels. Furthermore, the overexpression of SPARC in stably transfected 3T3-L1 cells induced insulin resistance and mitochondrial dysfunction (19). In this regard, Ghanemi et al concluded that Sparc-deficient mice had lower body weight, muscle, and white adipose tissue (20). Son et al also found that resistance training reduces SPARS (15). Nishida et al also showed that replacing sedentary time with moderate-to-vigorous physical activity is associated with a decrease in serum SPARC levels in middle-aged men (21). However, these results contradict the findings of Songsorn et al, indicating that intense and chronic exercise had no significant effect on women’s serum SPARS (22).

SPARC is involved in many processes such as wound healing, inflammation, extracellular protease activity, and angiogenesis. Changes in SPARC levels have been observed in many diseases such as osteoporosis, arthritis, cardiovascular diseases, obesity, and type 2 diabetes. It has been represented that there is an independent association between obesity and SPARC (23). In most obese people, the goal of physical activity is to increase energy expenditure, and this is directly related to the amount of muscle mass used during exercise. For this reason, an activity that involves more muscle mass will have the best results (23).

In addition, there is a possible relationship between exercise intensity and serum level or expression of

### Table 1. Primer sequence of genes used in research

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
</tr>
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<tbody>
<tr>
<td>GAPDH_F</td>
<td>AACCCTACCATCCTTCCAG</td>
</tr>
<tr>
<td>GAPDH_R</td>
<td>CCAGTAGACTCCAGACATAC</td>
</tr>
<tr>
<td>DECORIN_F</td>
<td>GAGTTGGGATATGGAGATGAAG</td>
</tr>
<tr>
<td>DECORIN_R</td>
<td>TGAGCGCTGTTGGGAGTTAC</td>
</tr>
<tr>
<td>SPARC_F</td>
<td>CAAGTCACACCATTTCCAG</td>
</tr>
<tr>
<td>SPARC_R</td>
<td>GCCTATACGCATCCGTTTCC</td>
</tr>
<tr>
<td>MYONECTIN_F</td>
<td>GGGTGAAGGCAAGATGACCTACAGG</td>
</tr>
<tr>
<td>MYONECTIN_R</td>
<td>TTGGGAAGTTTCTGCTAGAG</td>
</tr>
</tbody>
</table>
Aerobic and resistance training reduced SPARC and myonectin

SPARC (15). Exercise intensity, duration, and strength depend on physiological and biochemical parameters that limit exercise capacities, including oxygen saturation, lung capacity, heart condition, blood sugar, and physical disabilities. Indeed, exceeding these physiological limits will not only be harmful but also fail to provide the benefits of exercise (15).

In general, regular physical activity, if performed regularly and in the long term, can be effective in changing the level of SPARC and its expression. If the exercise intensity is more than average and more than 70% of VO2max and in resistance training, more than 70% of the maximum strength, due to the increase in glucose uptake by active tissues, an increase in the sympathetic activity of adrenal glands and energy expenditure, and glycogen depletion, along with the inhibition of glycolysis reduces SPARC (23).

On the other hand, our results revealed that a high-fat diet increased DCN, but this increase was not significant. Aerobic and resistance exercises had no significant effect on serum DCN expression. Despite its expression, it decreased slightly. In this regard, Nasiri et al reported that eight weeks of Pilates training had no significant impact on serum DCN (24). Ataeinosrat et al also concluded that low-intensity resistance training had no influence on serum DCN, but moderate and high-intensity training increased it in rats (25).

Our results confirmed that obesity increases myonectin, while aerobic and resistance exercises reduce it. Of course, resistance exercise caused a greater decrease in myonectin compared to aerobic exercise. Lim et al found that 10 weeks of aerobic exercise resulted in a significant decrease in myonectin levels in both young and old patients (26). Pourranjbar et al investigated the effect of aerobic exercise on myonectin serum levels and insulin resistance in obese and overweight women and reported that the myonectin serum level increased significantly in the experimental group (16). Peterson et al evaluated the effect of obesity and exercise on the expression of novel myokines, myonectin, and fibronectin type III domain containing 5 and demonstrated that exercise did not increase the expression of FNDC5 or myonectin gene (12).

Regarding the role of exercise in reducing myonectin, no clear mechanism can be mentioned, but some research has shown that reducing insulin resistance under the influence of exercise is effective in reducing myonectin. It seems that for a better effect of exercise, regardless of its type (aerobic or resistance), its duration and intensity should be longer to have a better effect on myokines. Additionally, factors such as gene expression or protein level, exercise intensity, and cell tissue for myokine investigation can influence the contradictory results of research with each other.
Conclusion
Aerobic and resistance exercise decreased myonectin and SPARC expressions in the skeletal muscle of rats fed with a high-fat diet, but it had no significant effect on the expression of DCN.

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Conceptualization: Mostafa Babaeinejad.
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Investigation: Mostafa Babaeinejad, Hasan Mateenhomaie.
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Supervision: Hasan Mateenhomaie, Hoseyn Fatolahi.
Writing–original draft: Mostafa Babaeinejad.
Writing–review & editing: Hasan Mateenhomaie, Hoseyn Fatolahi.

Competing Interests
The authors have no conflict of interests.

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