Journal of Vessels and Circulation
Qom University of Medical Sciences
Winter 2022. Volume 3. Number 1

Research Paper

# Effects of 8 Weeks Resistance Training on the Expression of MicroRNAs Associated With Adipose Tissue Angiogenesis in Rats on a High-Fat Diet

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**Please cite this article as** Sadeghian H, Siahkohian S, Akbarpour Beni M, Bolboli L. Effects of 8 Weeks Resistance Training on the Expression of MicroRNAs Associated With Adipose Tissue Angiogenesis in Rats on a High-Fat Diet. Journal of Vessels and Circulation. 2021; 3(1):17-26. http://dx.doi.org/10.32598/JVC.3.1.107.1

doi: http://dx.doi.org/10.32598/JVC.3.1.107.1



**Article info**
**Received:** 23 Feb 2022
**Accepted:** 03 Mar 2022
**Publish:** 01 January 2022

**Keywords:**
High-fat diet, Angiogenesis, MicroRNAs, Resistance training

## ABSTRACT

**Background and Aim:** Recent research has shown that microRNAs (miRNAs) can be regarded as new biomarkers for metabolic and angiogenesis-related diseases. This study aimed to investigate the effect of 8 weeks of resistance training on the expression of miRNAs associated with adipose tissue angiogenesis in rats with a high-fat diet.

**Materials and Methods:** Forty male Wistar rats were randomly divided into four groups (each with 10 rats): normal diet, normal diet+resistance training, high-fat diet, and high-fat diet+resistance training. The resistance training groups performed 5 sessions of resistance training protocol each week for 8 weeks. After one week of familiarity with ladders, in the 2nd week, weights of 30% of body weight were attached to the rats' tails, which gradually reached about 180% of their body weight by the end of 8 weeks. To evaluate the effect of resistance training and a high-fat diet on miR-221, miR-222, miR-329, and vascular endothelial growth factor (VEGF) variables, sampling of mesenteric adipose tissue was performed immediately after killing the animals.

**Results:** The levels of miR-221, miR-222, and VEGF in the resistance training group+normal diet and resistance training group+high-fat diet increased significantly compared to the control groups (P=0.001, and P=0.001, respectively). However, no significant difference was observed in any groups regarding miR-329 (P=0.103).

**Conclusion:** The results showed that resistance training increased microRNAs levels associated with adipose tissue angiogenesis in rats on a high-fat and normal diet.

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## 1. Introduction

Because of changes in lifestyle and consuming more high-calorie and high-fat foods, weight gain and obesity are seen more in society. Research has shown that eating high-fat foods increases the number and volume of adipose cells, which ultimately results in adipose tissue proliferation and various problems such as insulin resistance, high blood pressure, cardiovascular disease, metabolic syndrome, stroke, type 2 diabetes, and sudden death [1, 2]. For this reason, in recent years, researchers have investigated the relationship between a high-fat diet, adipose tissue, and vascular diseases [3, 4].

Adipose tissue needs angiogenesis for its survival and development, so it produces vascular endothelial growth factor (VEGF). On the other hand, increasing fat mass leads to the secretion of anti-angiogenesis factors, including resistin, and ultimately obesity leads to atherosclerosis and cardiovascular diseases. Therefore, adipose tissue survival depends on its vascular network [5, 6]. When new adipose tissue is made, the required blood vessels are also formed [7, 8]. Excessive expansion of adipose tissue can lead to hypoxia. A significant reduction (70%) in adipose tissue $PO_2$ was reported in obese rats. Factors such as leptin, adiponectin, VEGF, and hypoxia are involved in adipose tissue angiogenesis [9]. Stimulation of VEGF leads to the growth, migration, and survival of endothelial cells, resulting in further expansion of the vascular network and growth of the adipose mass, but extra stimulation of antiangiogenic factors versus angiogenic factors suppresses the process of angiogenesis; as a result, angiogenesis stops and the arteries are damaged [10].

Factors such as the type of diet, metabolic and hormonal diseases, as well as lifestyle, are effective in the process of angiogenesis. In other words, exercise, on the one hand, and obesity, on the other hand, change angiogenesis in adipose tissue, resulting in changes in metabolic processes [11, 12]. So far, few studies have been conducted on the effect of resistance exercise on the activity of visceral adipose tissue and subcutaneous fat, and most studies have examined the effect of endurance activities on adipokines and cytokines of adipose tissue. According to Disanzo et al. (2014), aerobic exercise increases the expression of the VEGF-A gene in adipose tissue, and this gene is a crucial factor in the angiogenesis of this tissue [13]. Molla Hassanzadeh et al. (2016) investigated the effect of 8 weeks of aerobic exercise on angiogenesis and body composition in overweight women. The results showed that 8 weeks of aerobic exercise increased VEGF and significantly reduced the body mass index of overweight women [14]. Van Pelt et al. (2017) also showed that aerobic exercise increased angiogenic factors in subcutaneous fat of overweight women [15]. Therefore, exercise is one of the appropriate and effective ways to regulate the metabolic status of the body. In addition, exercise can prevent metabolic and cardiovascular diseases not only by reducing fat mass but also by regulating transcription and improving protein levels. Furthermore, they prevent adipose tissue hypoxia by increasing mRNA and VEGF in adipose tissue [16].

Experts have drawn their attention to the effect of microRNAs (miRNAs) in various cellular and molecular processes. MicroRNAs are small fragments of RNA molecules, 18-22 nucleotides in length, that play an essential role in regulating the activity of genes and induce the degradation or inhibition of specific mRNA translation of target genes [17, 18]. miRNAs are transcribed in the nucleus as fragments of several hundred nucleotides. This precursor miRNA becomes an incomplete hairpin and matures into a mature miRNA during cutting and processing in the nucleus and cytoplasm [18]. Therefore, the role of miRNAs in angiogenesis in various tissues, including adipose tissue, can be mentioned. Some research has examined various microRNAs for angiogenesis: miR-93, miR-195, miR-276, miR-34a, miR-124, miR-329, miR-199b, miR-204, miR-200b, miR-361-5p, miR-874, miR-125-5p, miR-146, miR-211, and miR-222 [19, 20]. Based on studies on the expression and function of miRNA in angiogenesis, they fall into two major classes. One group of miRNAs, including miR-34a, miR-124, miR-29, miR-126, miR-150, miR-221, miR-222, and miR17-92 further regulate angiogenesis by targeting specific genes. The other group of miRNAs is modulated by antiangiogenic or hypoxic factors. They include miR-483-3p, miR-21, miR-210, miR-296, miR-93, miR-206, miR-26, miR-155, miR-424, miR-27b, and miR-130a [19].

Evidence shows that miR-221 and miR-222 have the same genes, target, and biological functions in physiological angiogenesis and tumor [21, 22]. However, an interesting study shows that these two miRNAs have different activities in mediating angiogenesis. miR-222 inhibits mediated vascular growth (cell proliferation and migration, transducer and transcription activator bound to 30UTR expression, and cyclin D1 in ECs) [23]. Thus, the role of miR-222 or its targets is cell-dependent. miR-221 and miR-222 have many mRNA targets responsible for miRNA-mediated angiogenesis [21]. miR-329 adversely regulates angiogenesis by directly targeting the angiogenesis CD146 gene. miR-329 expression is decreased by VEGF and TNF-α, leading to positive regulation of CD146 and increased angiogenesis [24].

Moderate-intensity aerobic exercise does not affect subcutaneous adipose tissue angiogenesis in individuals with insulin resistance. In this regard, it has been shown that intense interval training versus continuous training reduces visceral and subcutaneous fat. It has been suggested that further reduction of visceral and subcutaneous fat after strenuous interval training may be due to angiogenesis hormones. In general, exercise improves body composition and associated risks by reducing cell volume rather than cell count. Considering the effect of exercise on miRNAs related to adipose tissue and the contradictory results regarding the process of angiogenesis in adipose tissue and the role of a high-fat diet in its creation, we aimed to evaluate the effect of 8 weeks of resistance training on some of the microRNAs associated with adipose tissue angiogenesis in high-fat dietary rats.

## 2. Materials and Methods

Forty male 6-week-old Wistar rats (Mean±SD body weight: 193.38±10.85 g) were prepared from the Pasteur Institute of Iran. After transferring to an animal laboratory environment and a week of familiarity with the new environment, the animals were weighed with a digital scale (Rat grimace scale) with an accuracy of 0.0001 g and then randomly divided into two groups of normal diet (n=20) and high-fat diet (n=20) (1st phase). In the 2nd phase, the subjects of the high-fat diet group were fed a high-fat diet for four weeks. Then, after four weeks, the subjects in each group were randomly divided into the following two groups (n=10) (3rd phase).

The normal diet group was divided into two groups (n=10 per group): 1) normal diet and 2) normal diet+resistance training. The high-fat diet group was divided into two groups (n=10 per group): 1) high-fat diet and 2) high-fat diet+resistance training. The groups were homogenized based on the mean weight. The rats in the resistance training groups then performed their training protocol for 8 weeks. Normal and high-fat diet groups did not participate in any exercise program during this period (the 4th phase). Also, five rats lost their lives during the investigation for unknown reasons (two rats in the high-fat diet group and one rat in the other groups), and finally, 35 rats remained alive.

### To keep and feed the subjects

After transferring the subjects to the laboratory environment, they (in groups of 5) were kept in transparent polycarbonate cages at 20°C-24°C, a humidity of 45% to 55%, over a 12:12 hours light: dark cycle. During the study period, the group with a normal diet of standard mouse food and the group with a high-fat diet, according to the research of Srinivasan et al., were fed high-fat food (58% of calories in the form of fat) as pellets. A high-fat diet is a combination of normal rat meal (365 g/kg), pork fat (sheep fat used as a substitute in this study (310 g/kg), casein (250 g/kg), cholesterol (10 g/kg), mixed vitamins and minerals (60 g/kg), DL-methionine (3 g/kg), yeast powder (1 g/kg) and sodium chloride (1 g/kg) [25]. Subjects in the high-fat group and the high-fat diet group+resistance training began to eat a high-fat diet four weeks before the start of the training protocol. At the same time as the training protocol, all animals had free access to water and food.

### Resistance training protocol

Resistance training consisted of five sessions of climbing a 1-m ladder with 46 steps per week for 8 weeks. In this training method, after attaching the weight to the rat's tail, the rat was encouraged to climb a vertical ladder (90 degrees). A weight was attached by a narrow rope with tape to the end of the mouse tail (2 to 3 cm below the beginning of the tail) and was considered an overload. The weights included lead bags that were adjustable according to the target weight. After one week of familiarity with climbing ladders, in the 2nd week, a weight as heavy as 30% of their body weight was attached to the rat's tail, which gradually reached about 180% of their body weight [26]. The exercises were performed in 5 sets of 4 repetitions with 3 minutes of rest between sets and about 10 seconds of rest between repetitions (Table 1). Before starting each exercise program, they did two sets of five repetitions to warm up without weights. In the end, they did a 5-repetition set without weights to cool down [26, 27].

### Method of anesthetizing subjects, collecting, and preserving tissue

Subjects in each group were anesthetized 48 hours after the last exercise training session with a peritoneal injection of a combination of ketamine (70 mg/kg), and xylazine (3-5 mg/kg) were killed. A sampling of mesenteric (visceral) adipose tissue was performed immediately after killing the animal, and then 1 g of the tissue was fixed in a 10% formalin buffer (German Merck). The stabilized tissue was then dehydrated during various stages, placed in paraffin molds (Majal Company, Iran), and frozen in a nitrogen capsule at -80°C.

**Table 1.** Resistance training protocol on a 1-m ladder (Rest between sets=3 minutes)

| Week | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 7th |
|---|---|---|---|---|---|---|---|---|
| Intensity (percentage of body weight) | Getting to know | 30 | 70-80 | 100 | 120 | 140 | 160 | 180 |
| Set×repetition | | 5×4 | 5×4 | 5×4 | 5×4 | 5×4 | 5×4 | 5×4 |

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## Preparation of tissue extract and measurement of dependent variables

Frozen tissues were placed in a refrigerator full of liquid nitrogen and pounded when floating in the liquid. Before the resulting powder lost its state, 100 mg was rapidly put in a microtube, and 1 mL of saline phosphate buffer was added. Then it was placed on the shaker (IKA MS 3D digital model made in the USA) for 1 minute. According to the kits' instructions for 20 minutes with a rotation speed of 3000 rpm, a centrifuge with a solution of 15000 rpm was obtained with the help of a model centrifuge (FEF 1401). The centrifuge extract was refrozen and refrigerated to measure the research variables. VEGF measurement was done using the ELISA method and Kosa Bio Biotechnology Company kit for mouse samples according to the manufacturer's instructions (CUSABIO, China). The sensitivity of the method and the dispersion coefficient was measured as 0.033 ng/ml and 8%, respectively (sensitivity=0.033 ng/mL and $P_{Intrassay}$=8%). ELISA steps were performed to measure the dependent variable as follows. 1st, the standard solution was diluted based on the kit brochure. Then, 40 Landa from the tissue sample were combined with 10 Landa antibodies and, after half an hour, were washed with an automatic washer and kept at 37°C. Substrate mixtures A and B were added in equal proportions and incubated at 37°C without light. In the next step, 50 Landa were added from the stop solution. Then, with the help of an ELISA device, VEGF was determined according to the color change ratio created and in proportion to the generated wavelength reading.

## To remove RNA from sir samplers

All Sir Samples and microtubules used to extract microRNAs were placed overnight in DEPC solution at a concentration of one-tenth of a percent and then dried using an oven at 45°C; then samplers and microtubes were autoclaved twice regularly.

## Extraction of microRNAs

To extract microRNA, we used the RNA extraction kit (Rena Biotechnology Company), access code (RB1001), and according to the manufacturer's instructions. Also, RT steam-loop housekeeping method was used to evaluate microRNA levels. 1st, we mixed 50 mg of the tissue with 1 mL of bufferrazole to obtain a homogeneous solution and then transferred the mixture to a 2-mL microtube. The mixing solution was placed at a constant ambient temperature for 5 minutes. Then, 200 µL of chloroform was added to the set, shaken vigorously for 10-15 second, and left in the medium for 5 minutes. The tube containing the mix was centrifuged at 8000 rpm for 5 minutes. At the end of this phase, the two phases were visible. The clear supernatant containing the RNA was removed and transferred to another tube; 1000 µL of 100% cold ethanol was added and then placed at -20°C for 8 minutes. Finally, the tube was removed from the freezer and centrifuged. Similar to the previous step, the liquid inside the tube was discarded after centrifugation. At this stage, sometimes but not usually, colorless or whitish spots were observed on the body and bottom of the tube. The centrifuge was repeated by adding 80% ethanol for washing, and after removing the ethanol and drying the contents inside the tube (the air-dry method was used at this stage), 50 to 20 µL of double distilled water was added depending on the amount of precipitate and dissolved in the tube with an RNA pipette.

Next, 5 µL of the mixture was analyzed qualitatively using electrophoresis on 1% agarose gel to be used in cDNA synthesis after confirmation. For DNA replication, DNA strands were removed from the extracted RNA product using DNase I. For this purpose, after adding the DNase enzyme, the reaction mixture was incubated at 37°C for 30 minutes. To confirm the RNA quality, the decomposed DNA sample was analyzed on 1% agarose gel.

## miRNA synthesis

According to Table 2, cDNA synthesis was performed using an RNA biotechnology cDNA synthesis kit. All materials were provided by Rena Biotechnologists Company. After completing the process and obtaining the threshold cycles (ct), the expression of the desired variables was measured using mathematical calculations. ΔCt of each sample was calculated using an internal

**Table 2.** The sequence of microRNAs primers examined

| Primer Name | Primer Sequence From MiRBase Database |
|---|---|
| MiR-329 | GAGGUUUUCUGGGUUUCUGUUUC |
| MiR-221 | UAGCAGCACGUAAAUAUUGGCG |
| MiR-222 | UAGCAGCACAUAAUGGUUUGUG |
| MiR-139-5p | UCUACAGUGCACGUGUCUCCAGU |



control (miR-139-5p) and miRNA (subtracting Cts from internal control). Then, the corresponding ΔΔCt for each sample was calculated by subtracting the ΔCt of that sample from the mean ΔCt of the control group. Finally, a mathematical relation was used to report changes in the quantitative expression of variables (Table 2).

### Statistical method

Descriptive statistics indices (Mean±SD) were used to describe the variables. Regarding the inferential statistics section, the Shapiro-Wilk test was used to assess the normality of the data in the study groups. After proving the normality of the data, the independent t-test, One-way, and Two-way ANOVA were used to determine the differences between dependent variables in the research groups, and the Scheffe post hoc test was used to compare the two groups. The significance level of $P \leq 0.05$ was considered for all statistical tests. All calculations were performed in SPSS Software version 26.

## 3. Results

The study results did not show a significant difference between the initial average weight of the normal diet groups and the high-fat diet (t=0.75, P=0.45). While after 12 weeks, a significant difference in the subject's body weight was observed after the diet intervention in the group of a normal diet and high-fat diet (t=4.40, P=0.001). Table 3 presents the Mean±SD of the rat's body weight in each group in the initial weight stage, weight after 4 weeks, and weight after 12 weeks.

Table 4 presents the statistical indicators related to the research variables. To investigate the interactive effect of resistance training and high-fat diet on the research variables, a Two-way ANOVA was performed. The results showed that the interaction effect of exercise and diet on miR-222 was significant (P=0.045, F=4.374). This means that resistance training and diet significantly increased miR-222. The calculated effect size equals 0.12, and the test power to detect this difference was 0.52. However, the interaction of exercise and nutrition on miR-221 (P=0.061, F=3.785), miR-329 (P=0.334, F=1.123), and VEGF (P=0.249, F=1.379) is not significant (Table 5).

Also, the main effect of training on miR-221 (P=0.001, F=107.79), miR-222 (P=0.001, F=136.96), and VEGF (P=0.001, F=61.99) is significant. There is a significant difference between the exercise groups, but it is not significant on miR-329 (P=0.103, F=2.820). The study of the main effect of nutrition showed that the main effect of nutrition on VEGF (P=0.008, F=8.159) is significant.

**Table 3.** Distribution of body weight (g) in different groups

| Group | | No. | Mean±SD | | |
|---|---|---|---|---|---|
| | | | Initial Weight | Weight After 4 Weeks | Weight After 12 Weeks |
| Normal diet | Normal diet+Resistance training | 9 | 191.69±11.47 | 253.24±11.22 | 322.79±12.95 |
| | Normal diet | 9 | 189.10±7.64 | 247.42±9.55 | 352.27±11.63 |
| High-fat diet | High-fat diet+Resistance training | 9 | 192.63±15.53 | 267.64±9.02 | 348.65±9.42 |
| | High-fat diet | 8 | 193.92±9.60 | 270.88±9.42 | 409.35±10.36 |

**Table 4.** Statistical indicators related to miR-222, miR-221, VEGF, miR-329

| Group | | Mean±SD | | | |
|---|---|---|---|---|---|
| | | miR-221 | miR-222 | miR329 | VEGF |
| Normal diet | Normal diet+Resistance training | 1.993±0.62 | 3.477±0.95 | 0.353±0.08 | 3.079±0.88 |
| | Normal diet | 0.218±0.09 | 0.194±0.11 | 0.537±0.39 | 0.889±0.50 |
| High-Fat diet | High-fat diet+Resistance training | 2.095±0.42 | 3.228±0.89 | 0.582±0.24 | 2.103±0.93 |
| | High-fat diet | 0.880±0.36 | 0.940±0.46 | 0.781±0.49 | 0.482±0.28 |

Abbreviation: VEGF: Vascular endothelial growth factor.

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There was a significant difference between normal and high-fat diet groups, but on miR-221 (P=0.12, F=2.041), miR-222 (P=0.304, F=1.091), and miR-329 (P=0.056, F=4.226) is not significant (Table 5).

To compare 2 by 2, four groups with each other, the Scheffe post hoc test was used; the results of the post hoc test showed a significant difference between the normal food+resistance training group with the normal food group regarding miR-221 (P=0.001), miR-222 (P=0.001), and VEGF (P=0.001). Due to the differences in means, resistance training has significantly increased miR-221, miR-222, and VEGF levels in the normal food group+resistance training. There was also a significant difference between the normal diet+resistance training group and the high-fat diet in miR-221 (P=0.013), miR-222 (P=0.001), and VEGF (P=0.001). Due to the difference between the means of the groups, miR-221, miR-222, and VEGF levels in the high-fat food group are less than the normal diet+resistance training group.

In addition, a significant difference was observed between the high-fat group+resistance training food and the normal diet group in miR-221 (P=0.001) and miR-222 (P=0.001). According to the difference between the means, resistance training has significantly increased miR-221 and miR-222 levels in the high-fat diet+resistance training group. Also, a significant difference was observed between the high-fat

**Table 5.** Results of Two-way analysis of variance to investigate the effect of exercise and nutrition interventions on study variables

| Variables | Source | Sum of Squares | df | Mean of Squares | F | Sig. | Eta | Observed Power |
|---|---|---|---|---|---|---|---|---|
| miR-221 | Diet | 1.274 | 1 | 1.274 | 2.041 | 0. 120 | 0.085 | 0.329 |
| | Training | 19.512 | 1 | 19.512 | 107.799 | 0.000*** | 0.777 | 1.000 |
| | Diet×training | 0.685 | 1 | 0.685 | 3.785 | 0.061 | 0.109 | 0.470 |
| miR-222 | Diet | 0.540 | 1 | 0.540 | 1.091 | 0.304 | 0.034 | 0.173 |
| | Training | 67.728 | 1 | 67.728 | 136.962 | 0.000*** | 0.815 | 1.000 |
| | Diet×training | 2.163 | 1 | 2.163 | 4.374 | 0.045* | 0.124 | 0.527 |
| miR-329 | Diet | 0.489 | 1 | 0.489 | 4.226 | 0.056 | 0.122 | 0.522 |
| | Training | 0.319 | 1 | 0.319 | 2.820 | 0.103 | 0.083 | 0.370 |
| | Diet×training | 0.268 | 1 | 0.134 | 1.123 | 0.334 | 0.047 | 0.236 |
| VEGF | Diet | 4.171 | 1 | 4.171 | 8.159 | 0.008** | 0.208 | 0.790 |
| | Training | 31.693 | 1 | 31.693 | 61.994 | 0.000*** | 0.667 | 1.000 |
| | Diet×training | 0.705 | 1 | 0.705 | 1.379 | 0.249 | 0.043 | 0.207 |

*P<0.05. **P<0.01. ***P<0.001.

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diet+resistance training group and the high-fat diet group in miR-221 (P=0.004), miR-222 (P=0.001), and VEGF (P=0.007) levels. Due to the difference between the means of the groups, miR-221, miR-222, and VEGF levels in the high-fat diet group are less than the high-fat diet+resistance training group.

## 4. Discussion

The current study aimed to investigate the effect of 8 weeks of resistance training on the expression of miRNAs associated with adipose tissue angiogenesis in rats with a high-fat diet. The results showed that the interaction effect of exercise and a high-fat diet on miR-222 was significant, meaning that 8 weeks of resistance training and a high-fat diet caused a significant increase in miR-222 level compared to the control group. The study of the main effects of the variables showed that 8 weeks of a high-fat diet caused a significant decrease in VEGF level compared to the normal diet group. In addition, the study of the main effect of training showed that the effect of resistance training on miR-221, miR-222, and VEGF was significant, meaning that 8 weeks of resistance training increased these variables compared to the non-training group. However, the effect of resistance training on miR-329 was not significant and did not change this variable. The results of this study are consistent with the results of Schmitz et al. (2018), who examined longer work/rest intervals during high-intensity interval training (HIIT) at miR-222 levels [28]. Their results showed that HIIT training could increase the circulating levels of miR-222 related to heart growth. MiR-222 could be a useful marker for monitoring the overall HIIT response and identifying optimal work/rest combinations [28]. This finding is also consistent with observations from animal studies by Liu et al. (2015) on increased miR-222 levels in rats that performed aerobic exercise. Thus, ECs and cardiomyocytes appear to be the main source of increased miR-222 levels during HIIT, while natural killer cells may also play a role in this event [29].

The results also showed that resistance training increased VEGF in the training group compared to the control group, which is consistent with the results of Fernandes et al. (2018) and Disanzo et al. (2014) studies. They stated that aerobic exercise increases the expression of the VEGF-A gene in adipose tissue and is suggested as an important angiogenic factor and mechanism for potential therapeutic applications in vascular diseases [13, 30]. Therefore, it seems that the increase in VEGF levels due to exercise is an important factor in the development of angiogenesis in adipose tissue, which is an important factor and an advantage in athletes over non-athletes. Angiogenesis factors in adipose tissue are caused by hypoxia, which occurs due to increased fat mass. Stimulation of VEGF causes the growth, migration, and survival of endothelial cells, resulting in further expansion of the vascular network and growth of fat mass [10].

On the other hand, the results showed that resistance training increased miR-221 compared to the control group, which is contrary to the results of research by Lee et al. (2018). They investigated the dynamic regulation of circulating miRNAs during acute exercise and long-term training in basketball athletes [31]. This study showed for the 1st time that miR-146a, miR-21, miR-221, and miR-210 are reduced in response to acute exercise in basketball athletes. Reasons for this difference in results include differences in exercise protocol, different miRNAs in the blood and tissues of the body, as well as differences in subjects. Changes in miR-221 were significantly associated with peak workload and creatine kinase (after three months of basketball matches). These findings provide the basis for defining miRNAs as biomarkers and their physiological role in cardiovascular adaptation resulting from long-term exercise. By targeting multiple transcripts, miR-221 regulates multiple aspects of the cellular response to hypoxia, such as inhibition of apoptosis [32, 33], changes from mitochondrial respiration to glycolysis [34], and induces angiogenesis [32, 35]. Thus, a decrease in miR-221 may reflect an initial cellular response to hypoxia after acute exercises, such as an increase in cellular apoptosis and mitochondrial metabolism. In addition, the inflammatory response is another process associated with exercise, especially acute high-intensity exercise. Also, miR-21 [36], miR-221 [37], and miR-146a [38, 39] have been identified as critical dampers in the inflammatory response. It can be argued that the decrease in miR-21, miR-221, and miR-146a levels may reflect the initial inflammatory process that occurs after acute exercise [40]. In addition, the study results are contrary to the results of Akbarpour et al. (2020). They investigated the effect of 8 weeks of resistance training on some miRNAs associated with cell death in women with breast cancer. The results showed that 8 weeks of resistance training caused a significant increase in miR-329, miR-15, and miR-16 levels in women with breast cancer. As a result, 8 weeks of resistance training increases some of the microRNAs associated with cell death in women with breast cancer, which can increase cell death and decrease cancer mass in women with breast cancer [41]. Also, miR-329 negatively regulates angiogenesis by directly targeting the CD146 gene in the angiogenesis process. Besides, miR-329 expression is decreased by VEGF and TNF-α, leading to positive regulation of CD146 and increased angiogenesis [24].

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There is ample evidence that miR-221, and miR-222 have the same genes, targets, and similar biological functions in physiological angiogenesis [21, 22]. However, an interesting study showed that these two miRNAs have different activities in mediating angiogenesis. Also, miR-222 (but not miR-221) inhibits mediated vascular growth (including cell proliferation and migration, transducer and transcription activator bound to 30UTR expression, and cyclin D1 in ECs) [23]. Thus, the role of miR-222 or its targets is cell-dependent. It is also possible that the specific biological activity of miRNAs of miR-221 and miR-222 depends on the specific sequence differences downstream of the general grain, particularly nucleotides 13-16, in the two miRNAs [21]. Meanwhile, miR-221 and miR-222 have many mRNA targets responsible for miRNA-mediated angiogenesis. Therefore, more studies are needed on other unknown gene targets to fully understand their molecular mechanisms. In addition, miR-126 reduces endostar and regulates miR-221, thereby suppressing angiogenesis in human central venous endothelial cells. Endostar can inhibit the formation of new blood vessels in fat and normalize blood vessels in adipose tissue, which is also called the injection of recombinant human endostatin [16]. In a word, miR-221 and miR-222, with different biological activities, are completely involved in angiogenesis. These new findings could have far-reaching implications for treating various angiogenesis-related diseases. It is promising that other analyses of the miR-221 and miR-222 genes will offer a deeper insight into the complex miRNA network in future research. Further development of therapeutic innovations using miRNA may become an option for treating angiogenesis-related diseases such as cardiovascular disease or incurable cancers.

## 5. Conclusion

In general, the study results showed that resistance training by increasing miR-221, miR-222, and decreasing miR-329 leads to activating pathways that increase VEGF protein and ultimately increase angiogenesis in rats with a high-fat and normal diet. Therefore, these exercises can be appropriate and effective strategies for modulating the body's metabolic status. However, due to the novelty of the subject and our research on an animal model, we suggest that more research be done on the effect of exercise and especially resistance training on miRNAs related to angiogenesis and anti-angiogenesis of human adipose tissue to gain more accurate results and a full understanding of their molecular mechanisms.

## Ethical Considerations

### Compliance with ethical guidelines

Ethical principles of research in accordance with the principles of working with laboratory animals were approved by the Ethics Committee in Biomedical Research of University of Qom. (Ethical Code: IR.QOM.REC.1399.002).

### Funding

This study was financially supported by the University of Qom.

### Authors' contributions

All authors equally contributed to preparing this article.

### Conflict of interest

The authors declared no conflict of interest.

### Acknowledgments

We thank all the colleagues who helped us carry out this research.

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