Research in Molecular Medicine

Effect of two Exercise Training Protocols on miR-133a and Runx2 in High-Fat Diet and Streptozotocin-induced Diabetes

Maryam Asadi Farsani[1], Ebrahim Banitalebi[1*], Mohamad Faramarzi[2], Nuredin Bakhtiari[3], Mostafa Rahimi[1]

1. Department of Sports Sciences, Faculty of Literature and Humanities, Shahrekord University, Shahrekord, Iran.
2. Department of Exercise Physiology, Faculty of Sports Sciences, Isfahan University, Isfahan, Iran.
3. Department of Biochemistry, Faculty of Biological Sciences, North Branch of Tehran, Islamic Azad University, Tehran, Iran.

Use your device to scan and read the article online

Citation Asadi Farsani M, Banitalebi E, Faramarzi M, Bakhtiari N, Rahimi M. Comparing the Antidiabetic Effect of two Exercise Training Protocols on miR-133a and Runx2 in High-Fat Diet and Streptozotocin-induced Diabetes: An Experimental Study. Research in Molecular Medicine. 2021; 9(3):163-172. https://doi.org/10.32598/rmm.9.3.810.2

https://doi.org/10.32598/rmm.9.3.810.2

Article Type:
Research Paper

Article info:
Received: 05 May 2021
Revised: 19 Jun 2021
Accepted: 13 Jul 2021

Keywords:
Exercise, MicroRNA, Diabetes mellitus, Aging

ABSTRACT

Background: Type 2 Diabetes (T2D) in the elderly is an epidemic that significantly impacts global health. This experimental study aimed to compare the responses of microRNA-133a (miR-133a) in different tissues and of Runt-related transcription factor 2 (Runx2) in bone marrow tissue following resistance and endurance training in old rats with High-Fat Diet and Streptozotocin (HFD/STZ)-induced type 2 diabetes.

Materials and Methods: T2D was induced by HFD/low-dose STZ in 30 male Wistar rats (21-month-old, Mean±SD weight 418±43 g). The rats received HFD (55%, 31%, and 14% of energy from fat, carbohydrate, and protein, respectively; 5.2 kcal/g). The diets continued for eight weeks in both groups. Over week four, the rats in the group with HFD/STZ-induced T2D received treatment with low-dose STZ. After one week of familiarity with the laboratory environment, they were randomly divided into three groups: Diabetic Endurance Training (DET, n=10), Diabetic Resistance Training (DRT, n=10), and Diabetic Control (DC, n=10). The eight weeks of endurance training protocol comprised five sessions of moderate-intensity training (60%-75% velocity at maximal oxygen uptake (vVO_{2max}) and low intensity (30%-30% vVO_{2max}). In 60% Maximum Voluntary Carrying Capacity (MVCC), the resistance group climbed the ladder 14-20 times with 1-minute rest, five days a week.

Results: The results of the 1-way ANOVA test showed no significant change in serum miR-133 expression (P=0.411) and muscle tissue (P=0.077) following resistance and endurance training. However, significant differences were observed in bone marrow miR-133 expression (P=0.003) and Runx2 gene expression (P=0.002) between groups. Tukey's post hoc tests showed that the bone marrow miR-133 expression had a significant increase following eight weeks of resistance training compared to the endurance training (P=0.006) and control (P=0.002) groups, and bone marrow Runx2 gene expression in rats exposed to resistance training compared to the endurance training (P=0.044) and the control (P=0.018) groups.

Conclusion: It seems that longer periods of exercise are required for cellular changes in the metabolism of these tissues after these exercise protocols. This topic should be studied in future research.

*** Corresponding Author:**
Ebrahim Banitalebi, PhD.
Address: *Department of Sports Sciences, Faculty of Literature and Humanities, Shahrekord University, Shahrekord, Iran.*
Phone: *+98 (913) 2818216*
E-mail: *banitalebi@sku.ac.ir*

Introduction

As a common chronic disease in the elderly population, type 2 Diabetes (T2D) has a major impact on global health [1]. T2D has harmful effects on bone and increases the risk of bone fractures [2]. Bone Marrow Stromal Cells (BMSCs) are critical cells for tissue engineering and regenerative medicine of various tissues because of their osteogenic differentiation capacity [3].

Historically, the mechanical interaction between bone and muscle has been well described, but in the last decade, the concept of muscle-bone crosstalk has emerged. Bone changes occurring due to alterations in muscle mass have been mainly attributed to biomechanical stimuli and compressive and tensile strengths applied to the skeleton [4]. On the other hand, skeletal muscle is an endocrine tissue capable of affecting metabolism in other tissues and organs through the secretion of hormonal factors (myokines) [5].

Muscle-specific microRNAs (myomiRs) usually control the fate of myogenic precursors and muscle homeostasis and regulate basic biological processes in muscles, including muscle remodeling, metabolism, and recovery [6, 7]. Previous studies documented the pivotal role of myomiRs in bone remodeling and homeostasis and the close association between impairment in some serum myomiRs and bone diseases [8]. For example, Cao et al. found an upregulation for miR-133a in circulating monocytes extracted from patients with postmenopausal osteoporosis [9]. MyomiRs are also involved in the pathophysiology of T2D [10]. In addition, it has been suggested that myomiRs could act as "endocrine signals" during myogenesis [11]. In this regard, miR-133a has been shown to play an essential role in fracture healing inhibiting through targeting Runt-related transcription factor 2 (Runx2) and several other molecules involved in osteoblastic differentiation and bone formation. Runx2, as a core-binding factor, is a specific transcription factor to regulate the differentiation of mesenchymal stem cells into osteoblasts. On the other hand, miR-133a in BMSCs reportedly inhibits bone resorption [12].

Further increase in muscle strength with resistance training helps older people improve sedentary life more effectively, which is a risk factor for T2D, possibly due to increased mitochondrial fatty acid β-oxidation. In addition, resistance training has improved hemoglobin A1c levels in the elderly [13].

Scientific research has shown that physical activity affects many molecular and cellular processes. Numerous factors such as mode, intensity, and duration of exercise training may affect the response of bone hemostasis indicators to exercise interventions [14].

We hypothesized that after endurance and resistance training, myomiRs could act as an endocrine factor and affect biomarkers of differentiation of bone marrow stem cells through blood circulation. Thus, the present study aimed to compare the response of miR-133a and Runx2 following resistance and endurance training in old rats with a High-Fat Diet and Streptozotocin (HFD/STZ)-induced type 2 diabetes.

Materials and Methods

This study was approved by the Local Ethics Committee for Laboratory Animals of Shahrekord University (Shahrekord City, Iran).

All procedures were performed according to the relevant instructions and regulations. This study was conducted according to ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) [15] for the care and use of research animals.

The research units were 30 male Wistar rats (21-month-old) purchased from Pasteur Institute of Iran (Karaj City, Iran), which were transferred to the laboratory animal housing of Shahrekord University to adapt to the Lab environment. The animals were kept at 22±3°C under a 12/12 h light/dark cycle with free access to rat feed and water. After one week of familiarity with the laboratory environment, the rats were randomly assigned to three groups: Diabetic Endurance Training (DET, n=10), Diabetic Resistance Training (DRT, n=10), and Diabetic Control (DC, n=10). Then, they did strength and endurance training protocols five sessions a week for eight weeks. Due to the adaptation of animals to training, they were tested for Maximum Voluntary Carrying Capacity (MVCC) once at the end of every four weeks. Endurance exercise intensity was determined based on a new exhaust test, the velocity at maximal oxygen uptake (vVO_{2max}) [16]. Next, 72 hours after the last training session, the animals were anesthetized by ketamine/xylazine combination for surgery, tissue extraction, and cellular-molecular tests and finally were dissected. It should be noted that seven rats failed to continue training during the study for various reasons, including failure to perform exercises properly or injury and illness. In the end, the animals were reduced to 7 in the endurance training group, 8 in the resistance training group, and 8 in the control group (Figure 1).

HFD/STZ-induced T2D

T2D was induced by HFD/low-dose STZ (manufactured by the Sigma Company) based on the protocol proposed by Zhang et al. [17] and Liu et al. [18]. The rats in the HFD group received 55%, 31%, and 14% of energy from fat, carbohydrate, and protein, respectively; 5.2 kcal/g. The diets continued for eight weeks for three groups. Over week four, the rats in the group with HFD/STZ-induced T2D received treatment with low-dose STZ (Sigma-Aldrich, St Louis, MO, USA). All rats received an intraperitoneal injection of low-dose STZ (30 mg/kg, dissolved in 0.1 M sodium citrate buffer at pH 4.4). One week later, blood glucose tests were prepared using a blood glucometer. The animals with blood glucose levels lower than 16.7 mmol/L received a second injection of STZ (30 mg/kg). These diets continued after injections. Four weeks after the injections, the animals with blood glucose concentrations over 16.7 mmol/L were regarded to have diabetes and chosen for more examinations [3, 19].

Determining velocity at maximal oxygen uptake (vVO_{2max})

The rodent treadmill was used to evaluate vVO_{2max} through ten 3-min stages of running the experiment. According to Leandro et al., the initial running speed test was 0.3 km/h, and a 0.3 km/h increase in the speed was considered in each 3-min period (with a slope of 0%). When the rats could not keep running, the vVO_{2max} was taken into account [20].

Identifying Maximal Voluntary Carrying Capacity (MVCC)

The familiarization of the rats was first carried out using a vertical climbing model (110 cm, 2-cm grid, 85o of inclination) with no overloads [21]. Overall, 72 h after the final session on familiarizing the rats, each animal received a test to assess Maximal Voluntary Carrying Capacity (MVCC). Regarding the primary climb, each rat was loaded 75% of its body weight carried to the top of the ladder (that is, the house chamber), and the rats could take a rest for a 120-s interval. Then, some weight increments of 30 g were added until the rats could not reach the top of the ladder because of the loads. This process was repeated until the animals failed in climbing the complete length of the ladder on three successive voluntary efforts. Besides, MVCC indicated the highest load, carried across the total length of the ladder successfully [21].

Resistance and endurance training protocols

The rats in the resistance training group performed a moderate-intensity ladder-based resistance training model to be familiarized with performing the training protocol for one week. After the last adaptation session, the MVCC test was taken from the animals, and MVCC was defined as the highest successfully carried weight [22]. According to the adaptation of animals to exercise at the end of the fourth week, the MVCC test was taken from the animals, and their exercise intensity was determined based on the new test [16, 23]. The moderate-intensity resistance training protocol consisted of climbing a special training ladder (110 cm long, 80° slope, 26 steps, and 2-cm space between each step) for eight weeks, five days a week. The moderate-intensity resistance training was performed with 60% of MVCC and 14-20 times climbing the ladder [16, 23].

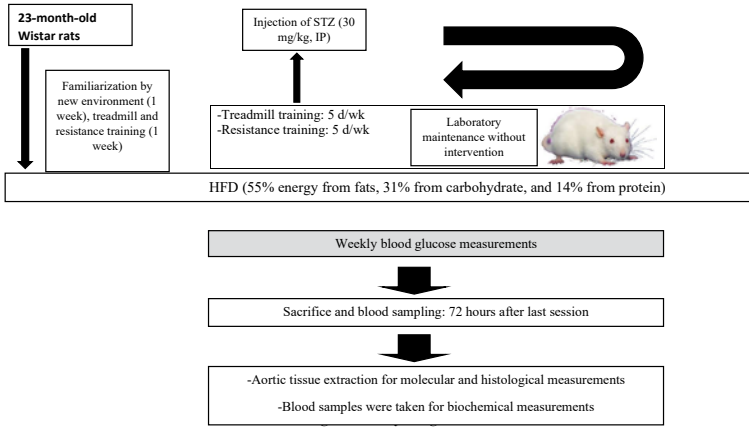

Figure 1. Study design

The animals in the endurance training group were introduced to the treadmill and how to run on it for a week. After 48 hours of rest from the last familiarization session, exhaustive testing was taken from the animals to determine exercise intensity [20]. The rodent treadmill was used to evaluate vVO_{2max} through ten 3-min stages of running the experiment. According to Leandro et al., the initial running speed test was 0.3 km/h, and a 0.3 km/h increase in the speed was considered in each 3-min period (with a slope of 0%). When the rats could not keep running, the vVO_{2max} was taken into account [20].

During the eight weeks of training, the animals were exposed to endurance training programs. The intensity of endurance training was then calculated by vVO_{2max}. At the beginning of ET, the animals were trained at 40%-50% vVO_{2max} for 5 minutes and a 0% slope to warm up. Endurance training protocol included frequent sessions of high-intensity training as well as low-intensity training, 2 minutes of running with 60% vVO_{2max} in the first week, 65% vVO_{2max} in the second week, 70% vVO_{2max} in the third week, and finally 75% vVO_{2max} in the fourth week until the end of training time. In addition, low-intensity competitions included a 2-minute run with 40% vVO_{2max} from the first week to the end of the third week and 30% vVO_{2max} from the beginning of the fourth week to the end of the eighth week. Finally, the number of high-intensity intervals increases from two to eight repetitions from the first week to the end of the eighth week [24].

Plasma glucose and insulin measurements

Blood glucose was determined by a glucometer (Germany) and enzyme-linked immunosorbent assay (ELISA) (Pars Azmoon, Cat. No: 5825; Tehran, Iran), with a sensitivity of 0.1 mg/dL in the tail-vein blood sample. After the induction of T2D with HFD/STZ, the measurement of the blood glucose levels was carried out to evaluate the start of hyperglycemic conditions (FBG>200 mg/dL). The measurement of the plasma insulin levels also took place through an ELISA kit (Monobind Co., Cat No: 5825-300; California, The USA) in animals subjected to fasting for 4 h.

RNA extraction

To this end, 72 hours after the last training session, the rats were anesthetized by intraperitoneal injection of ketamine (30 to 50 mg/kg) and xylazine (10 mg/kg). Then, bone marrow samples from the tibia and muscle tissue from Flexor Hallucis Longus (FHL) were harvested and collected into RNase-free microtubes. Finally, the samples were frozen in a nitrogen tank at -80°C and transferred to the laboratory [25].

Total Ribonucleic Acid (RNA) (here mRNA), as well as microRNA (miRNA), from FHL muscle and Bone Marrow (BM) via RNX-Plus solution kit (CinnaGen, Iran, Cat. No: RN7713C) and miR kit-amp Pars Genome (Pars Genome Company, Iran) were assessed according to the protocols provided by the manufacturers. A260 / A280> 2.0, A260 / A230> 1.8, NanoDrop 1000 (Thermo Scientific, Wilmington DE 19810 USA) were also used to measure the content and purity of RNA samples. In addition, integrity was determined using Ethidium Bromide (EtBr)-agarose gel electrophoresis (Biotium, Hayward, California, USA).

cDNA synthesis

RNA (5-10 µg) or mRNA (10-500 ng) transcription into complementary DNA (cDNA) was also done employing the RevertAid M-MuLV Reverse Transcriptase (M-MuLV RT) (1 µL) (Fermentas, GmbH, Germany), Deoxyribonuclease I (DNase I) (1 µL) (Thermo Scientific Co., Cat number: 89836), random hexamer primers (1 µL) (Thermo Scientific Co., Cat number: SO142), deoxynucleoside triphosphate (dNTP) (Thermo Scientific Co., Cat number: R0191) (2 µL), and RiboLock RNase inhibitor (Thermo Scientific Co., Cat number: N8080119) (0.25 µL) for 10 min at 25°C, followed by 60 min at 42°C in a final volume of 20 µL. The reaction was then halted for 5 minutes by raising the temperature to 70°C. In addition, cDNA synthesis in miRNA samples was performed based on the miR-amp kit (Cat No. 00101005, ParsGenome, Iran).

Real-Time Polymerase Chain Reaction (RT-PCR) was performed using the SYBR Green RT-PCR master mix kit (Qiagen, GmbH, Germany, Cat Number: 204052) to quantify mRNA. The expression of miRNA and mRNA was also normalized to the housekeeping gene Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH). The sequence of mRNA-specific primers and miRNAs is shown in Table 1. The RT-PCR protocol was performed in three versions on the Corbett RT-PCR (Rotor Gene-3000 Thermo Cycler) following a three-step program:

First, the samples were denatured at 95°C for 10 minutes. The amplification program was then completed in three steps (starting at 95°C for 15 s, then at 60°C for 30 s, and finally at 72°C for another 30 s). This procedure was generally repeated 40 times. In the end, the melting curve was analyzed in a cycle at a temperature between 72°C and 95°C (transfer rate at 1°C for 5 s).

Following the 2-ΔΔCT method, the relative gene expression for each gene was initially determined according

to its threshold cycle (CT). Then their CTs were compared to that of the GAPDH as the housekeeping gene [26].

Statistical analysis

The statistical analyses were performed using the SPSS software v. 21. Data normality was assessed by the Kolmogorov-Smirnov test, and homogeneity of variance was determined by Mauchly's sphericity test. For data with normal distribution, significant differences in levels between the three groups were investigated by 1-way analysis of variance (ANOVA), and the between-group differences were determined by Tukey's post hoc test. The significance level was set at $P \leq 0.05$.

Results

The results of the 1-way ANOVA test showed a significant difference between groups in blood glucose changes (P=0.002). But there were no significant differences in body mass (P=0.251) and insulin changes (P=0.121). Tukey's post hoc test for glucose changes showed a significant decrease in DRT (P=0.009) and DET (P=0.012) groups compared to the DC group. There were no significant differences between resistance training and endurance training groups (P=0.656) (Table 1).

The results of the 1-way ANOVA test showed no significant change in serum miR-133 expression (P=0.411) and skeletal muscle tissue (P=0.077) following DRT and DET. However, significant differences were observed in bone marrow miR-133 expression (P=0.003) and Runx2 gene expression (P=0.002) between groups. Tukey's post hoc tests showed that the bone marrow miR-133 expression had a significant increase following eight weeks of DRT compared to the DET (P=0.006) and DC (P=0.002) groups, and bone marrow Runx2 gene expression in rats exposed to DRT compared to the DET (P=0.044) and the DC (P=0.018) groups (Figure 2).

Discussion

Understanding the expression of miR-133 in serum, skeletal muscle, and bone marrow tissues and the Runx2 signaling and responses to various exercise protocols has become part of extensive research in old diabetic animals. Based on the literature review, no studies have evaluated the miRNA profile after endurance/resistance training to date. We were the first researchers who investigated the effects of chronic endurance/resistance training on the miR-133 profile of different tissues.

In the present study, we evaluated the effects of endurance and resistance training on miR-133 in serum, skeletal muscle, and bone marrow tissues and Runx2 signaling in bone marrow tissue of male Wistar rats with HFD/STZ-induced T2D. All data confirmed that daily food intake did not differ between groups. Due to the high risk of life-threatening T2D in elderly patients, exercise training has always been challenging to treat osteosarcopenia. Exercise training as a safe method can compensate for age-related bone and muscle disorders such as osteoporosis and sarcopenia [27].

The results indicated no significant changes in serum and skeletal muscle miR-133a expression following two exercise training protocols, but a considerable increase was observed in bone marrow miR-133a expression. One of the possible reasons for insignificant differences in the variables of the present study can be attributed to the short duration of the study. Most studies observing changes in variables lasted between 18 and 208 weeks

Table 1. Changes in weight, glucose, and insulin of diabetic rats after 8 weeks of resistance training

Variables	Time	Mean±SD			P
		Groups			
		RT	ET	DC	
Weight (g)	Pre-test	438.00±54.41	415.00±30.02	422.40±72.98	0.251
	Post-test	395.20±43.45	373.80±37.29	378.00±99.58	
Glucose (mg/dL)	Pre-test	458.83±102.99	358.55±64.18	376.80±107.98	0.002*
	Post-test	371.25±85.64	281.73±58.30	362.54±67.92	
Insulin (ng/mL)	Post-test	0.53±0.60	0.53±0.58	0.22±0.18	0.121

DC: Diabetic Control; DET: Diabetic+Endurance Training; DRT: Diabetic+Resistance Training.

*Significant change compared to the control group.

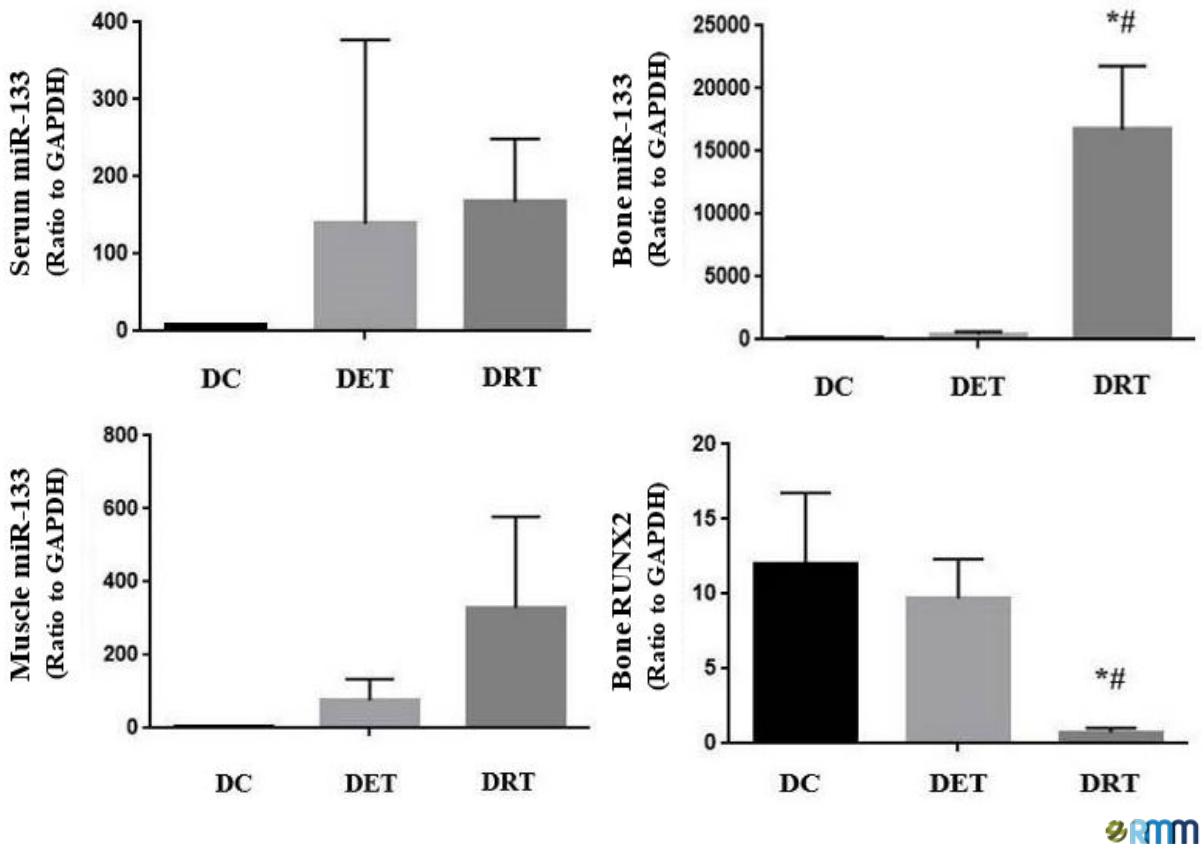

Figure 2. Changes in miR-133 expression level in serum, bone, and muscle tissues and in Runx2 expression level in bone tissue of diabetic rats after 8 weeks of resistance training

* Significant change compared to the control group. # Significant change compared to the endurance training group.

DC: Diabetic Control; DET: Diabetic+Endurance Training; DRT: Diabetic+Resistance Training; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase); Runx2: Runt-related transcription factor 2

[28], and even this response in older people needed more time than in young and adult people [29].

The miRNAs, which are expressed in various tissues and are highly stable, have been suggested as potential new biomarkers of sports-specific responses. In our study, bone marrow myomiRs-133a showed dynamic changes in response to resistance training, whereas serum and muscle miR-133a did not respond to these exercise protocols. Such differential changes for these myomiRs in serum/muscle/bone marrow tissues may indicate varying degrees of muscle fiber involvement or stress/adaptation in response to resistance training compared with endurance training [30].

The present study aimed to investigate whether miR-133a correlates with response to resistance/endurance training. The miR-133a is a key mediator in response to acute and chronic exercise. We found that the bone marrow miR-133a gene expression relative to muscle and serum tissues, as well as osteoblastic differentiation such as Runx2, differed significantly between the resistance and endurance training groups studied after eight weeks. The very low responses of miR-133a expression to endurance training are not surprising. However, several explanations may exist. A potential explanation could be the selective release of miR-133a by skeletal muscles, bone marrow, and serum in response to various exercise protocols [31]. Thus, unchanged expression of this microRNA in tissues undergoing endurance training may be partly due to the limited release of miR-133a by skeletal muscles and bone marrow in response to endurance training. Second, our analysis suggested that some myomiRs may play a role in muscle phenotypic changes and express intergroup changes in response to exercise training. Previous studies have evaluated the response to different exercise training of some myomiRs and microRNAs in the fine-tuning regulation of osteogenesis (osteomiRs) similar to the present study. These results are inconsistent with published studies and suggest that some of these osteomiRs [32] and myomiRs have changed in response to physical activities.

According to the present study's findings, endurance training did not improve serum/skeletal muscle/bone marrow miR-133a contents compared to other groups. The skeletal muscle levels of myomiRs-133a also experienced dynamic alterations in reaction to resistance training, whereas the serum/skeletal muscle/bone marrow miR-133a showed no responses to other treatments. These differential alterations in such myomiRs in muscle tissues could show various degrees of recruiting muscle fibers or stress/adaptation in reaction to resistance training compared with endurance training [30].

Of note, miR-133a is a crucial mediator of acute and chronic exercise responses. In this study, it was observed that gene expression of miR-133a in muscles, as well as osteoblast differentiation biomarkers such as Runx2, were significantly different between the resistance training group after eight weeks. The very low responses of serum/bone marrow miR-133a expression to endurance training were not surprising. However, there are several possible explanations, including the short study duration and the age of the rats.

These differential alterations experienced by the myomiRs in skeletal muscle tissues can show various levels of recruiting muscle fibers or stress/adaptation in reaction to resistance training compared with endurance training [30]. According to the primary hypothesis in this study, several circulating miRNAs, in particular myomiR-133a, were highly responsive to resistance training due to recruiting skeletal muscle over resistance training. Nevertheless, the analyses did not indicate any alterations in the mirR-133a levels of serum/skeletal muscle/bone marrow following endurance training. Accordingly, no secretion of muscle-specific miRNAs can be assumed into the blood through passive transport. In contrast, several miRNAs can have the potential of selective release, exerting the desired roles in different tissues or cells [33].

Another potential explanation could be the limited release of miR-133a by the skeletal muscle and the bone marrow to the serum in response to different exercise training [34, 35]. Thus, the unchanged expression of these microRNA in tissues with endurance training may be partly due to the limited release of miR-133a by the skeletal muscle and bone marrow tissues in response to the mentioned treatments. Thirdly, the analyses indicated that some myomiRs could conceivably play a part in the phenotypic changes of skeletal muscle and bone marrow and lead to pronounced intergroup variations in the endurance training response [36]. Previous studies have further evaluated the response to different types of endurance training in some of the same myomiRs and osteomiRs in the present study. These results did not correspond to the reports in the related studies, indicating that some of these ostemiRs [32, 37] and myomiRs [37-39] have changed in reaction to endurance training regimes skeletal muscle and bone marrow. Likewise, Nielsen et al. reported that 12 weeks of endurance training had decreased the miR-133a expression [38].

Another possible explanation was that endurance training regimens might reduce age-related differences in miRNA expression in skeletal muscle and bone marrow. However, adaptations due to muscle contraction in gene expression may differ between young and old animals [40, 41]. The altered gene expression pattern induced by endurance training regimens in old age does not improve miRNA expression in skeletal muscle and bone marrow. However, evidence suggests that muscles and bones are reactive as mechanical tissues [42]. Younger animals appear to be more mechanically responsive to myogenic and osteogenic signals, hormones, growth factors, and cytokines than older animals [43, 44].

It was also hypothesized that myomiRs might differentiate osteoblastic BMSCs in response to eight weeks of HFD/STZ-induced T2D endurance training. In addition, myomiRs isolated from skeletal muscle specimens may be involved in bone and muscle health benefits. More recently, experiments have shown possible patterns in cell-cell communication, where myomiRs may mediate cellular-molecular signaling in target cells similar to hormonal and myokine signaling [11, 45]. As the researchers found, myomiRs can be vital mediators of bone health, and exercise techniques may be responsible for cellular communication [46, 47].

Conclusion

In this study, resistance training exhibited higher osteogenic potential due to increased Runx2 expression. Thus, it could be concluded that resistance exercise has more potential for osteogenesis. But, cellular changes in the metabolism of these tissues after these exercises require longer periods of exercise, which should be studied in future research. However, some limitations should be noted about the present study. The method used in our study can only analyze a small number of myomiRs and osteomiRs expressed in FHL muscle and tibial-derived bone marrow, respectively. It means that we could not use other genome-wide association studies (such as sequencing and microarray). In addition, only older rats were examined for different physical training protocols. Future research should compare the effects of these pro-

tocols on the expression of myomiRs and osteomiRs and their association between young and old animals.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by Shahrekord University Local Ethics Committee for Laboratory Animals (Shahrekord, Iran). The procedures were performed in accordance with the relevant instructions and regulations. This experiment was performed according to the ARRIVE (Animal Research) guidelines for the care and use of research animals.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Study design: Maryam Asadi Farsani, Ebrahim Banitalebi, Mohammad Faramarzi, and Nuredin Bakhtiari; Supervision of the exercise training protocols: Maryam Asadi Farsani, Ebrahim Banitalebi, Mohammad Faramarzi, and Mostafa Rahimi; Supervision over laboratory experiments and the collection of the required data: Maryam Asadi Farsani, Ebrahim Banitalebi, Mohammad Faramarzi, and Mostafa Rahimi; Performing data analysis and interpretations: Maryam Asadi Farsani and Ebrahim Banitalebi; Writing the first draft of the manuscript: Maryam Asadi Farsani and Ebrahim Banitalebi; Editing the article: Maryam Asadi Farsani, Ebrahim Banitalebi, Mohammad Faramarzi, Nuredin Bakhtiari, and Mostafa Rahimi; Developing the study, reviewing and confirming the final draft: All the authors.

Conflict of interest

The authors declared no conflict of interest.

References

[1] Gambert SR, Pinkstaff S. Emerging epidemic: Diabetes in older adults: Demography, economic impact, and pathophysiology. Diabetes Spectr. 2006; 19(4):221-8. [DOI:10.2337/diaspect.19.4.221]

[2] Schwartz AV, Sellmeyer DE, Ensrud KE, Cauley JA, Tabor HK, Schreiner PJ, et al. Older women with diabetes have an increased risk of fracture: A prospective study. J Clin Endocrinol Metab. 2001; 86(1):32-8. [DOI:10.1210/jcem.86.1.7139] [PMID]

[3] Qian C, Zhu C, Yu W, Jiang X, Zhang F. High-fat diet/low-dose streptozotocin-induced type 2 diabetes in rats impacts osteogenesis and Wnt signaling in bone marrow stromal cells. PLoS One. 2015; 10(8):e0136390. [DOI:10.1371/journal.pone.0136390] [PMID] [PMCID]

[4] Cianferotti L, Brandi ML. Muscle-bone interactions: Basic and clinical aspects. Endocrine. 2014; 45(2):165-77. [DOI:10.1007/s12020-013-0026-8] [PMID]

[5] Banitalebi E, Kazemi A, Faramarzi M, Nasiri S, Haghighi MM. Effects of sprint interval or combined aerobic and resistance training on myokines in overweight women with type 2 diabetes: A randomized controlled trial. Life Sci. 2019; 217:101-9. [DOI:10.1016/j.lfs.2018.11.062] [PMID]

[6] McCarthy JJ. The MyomiR network in skeletal muscle plasticity. Exerc Sport Sci Rev. 2011; 39(3):150-4. [DOI:10.1097/JES.0b013e31821c01e1] [PMID] [PMCID]

[7] Güller I, Russell AP. MicroRNAs in skeletal muscle: Their role and regulation in development, disease and function. J Physiol. 2010; 588(Pt 21):4075-87. [DOI:10.1113/jphysiol.2010.194175] [PMID] [PMCID]

[8] Weilner S, Skalicky S, Salzer B, Keider V, Wagner M, Hildner F, et al. Differentially circulating miRNAs after recent osteoporotic fractures can influence osteogenic differentiation. Bone. 2015; 79:43-51. [DOI:10.1016/j.bone.2015.05.027] [PMID]

[9] Cao Z, Moore BT, Wang Y, Peng XH, Lappe JM, Recker RR, et al. MiR-422a as a potential cellular microRNA biomarker for postmenopausal osteoporosis. PloS One. 2014; 9(5):e97098. [DOI:10.1371/journal.pone.0097098] [PMID] [PMCID]

[10] Frias Fde T, de Mendonça M, Martins AR, Gindro AF, Cogliati B, Curi R, et al. MyomiRs as markers of insulin resistance and decreased myogenesis in skeletal muscle of diet-induced obese mice. Front Endocrinol (Lausanne). 2016; 7: 76. [DOI:10.3389/fendo.2016.00076] [PMID] [PMCID]

[11] Forterre A, Jalabert A, Chikh K, Pesenti S, Euthine V, Granjon A, et al. Myotube-derived exosomal miRNAs downregulate Sirtuin1 in myoblasts during muscle cell differentiation. Cell Cycle. 2014; 13(1):78-89. [PMID] [PMCID]

[12] Peng H, Lu SL, Bai Y, Fang X, Huang H, Zhuang XQ. MiR-133a inhibits fracture healing via targeting RUNX2/BMP2. Eur Rev Med Pharmacol Sci. 2018; 22(9):2519-26. [PMID]

[13] Lee J, Kim D, Kim C. Resistance training for glycemic control, muscular strength, and lean body mass in old type 2 diabetic patients: A meta-analysis. Diabetes Ther. 2017; 8(3):459-73. [DOI:10.1007/s13300-017-0258-3] [PMID] [PMCID]

[14] Honda A, Sogo N, Nagasawa S, Shimizu T, Umemura Y. High-impact exercise strengthens bone in osteopenic ovariectomized rats with the same outcome as Sham rats. J Appl Physiol (1985). 2003; 95(3):1032-7. [DOI:10.1152/japplphysiol.00781.2002] [PMID]

[15] Karp NA, Meehan TF, Morgan H, Mason JC, Blake A, Kurbatova N, et al. Applying the ARRIVE guidelines to an in vivo database. PLoS Biol. 2015; 13(5):e1002151. [DOI:10.1371/journal.pbio.1002151] [PMID] [PMCID]

[16] Krug AL, Macedo AG, Zago AS, Rush JW, Santos CF, Amaral SL. High-intensity resistance training attenuates dexamethasone-induced muscle atrophy. Muscle Nerve. 2016; 53(5):779-88. [DOI:10.1002/mus.24906] [PMID]

[17] Zhang M, Lv XY, Li J, Xu ZG, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin

induced type 2 diabetes rat model. Exp Diabetes Res. 2008; . 2008:704045. [DOI:10.1155/2008/704045] [PMID] [PMCID]

[18] Liu Z, Li W, Li X, Zhang M, Chen L, Zheng YN, et al. Antidiabetic effects of malonyl ginsenosides from Panax ginseng on type 2 diabetic rats induced by high-fat diet and streptozotocin. J Ethnopharmacol. 2013; 145(1):233-40. [DOI:10.1016/j.jep.2012.10.058] [PMID]

[19] de Bem GF, Costa CA, Santos IB, Cristino Cordeiro VDS, de Carvalho LCRM, de Souza MAV, et al. Antidiabetic effect of Euterpe oleracea Mart.(açai) extract and exercise training on high-fat diet and streptozotocin-induced diabetic rats: A positive interaction. PLoS One. 2018; 13(6):e0199207. [DOI:10.1371/journal.pone.0199207] [PMID] [PMCID]

[20] Leandro CG, Levada AC, Hirabara SM, Manhães-de-Castro R, De-Castro CB, Curi R, et al. A program of moderate physical training for Wistar rats based on maximal oxygen consumption. J Strength Cond Res. 2007; 21(3):751-6. [PMID]

[21] Singulani MP, Stringhetta-Garcia CT, Santos LF, Morais SR, Louzada MJ, Oliveira SH, et al. Effects of strength training on osteogenic differentiation and bone strength in aging female Wistar rats. Sci Rep. 2017; 7:42878. [DOI:10.1038/srep42878] [PMID] [PMCID]

[22] de Cássia Marqueti R, Almeida JA, Nakagaki WR, Guzzoni V, Boghi F, Renner A, et al. Resistance training minimizes the biomechanical effects of aging in three different rat tendons. J Biomech. 2017; 53:29-35. [DOI:10.1016/j.jbiomech.2016.12.029] [PMID]

[23] Macedo AG, Krug AL, Herrera NA, Zago AS, Rush JW, Amaral SL. Low-intensity resistance training attenuates dexamethasone-induced atrophy in the flexor hallucis longus muscle. J Steroid Biochem Mol Biol. 2014; 143:357-64. [DOI:10.1016/j.jsbmb.2014.05.010] [PMID]

[24] Nourshahi M, Hedayati M, Nemati J, Ranjbar K, Gholamali M. [Effect of 8 weeks endurance training on serum vascular endothelial growth factor and endostatin in wistar rats (Persian)]. Koomesh. 2012; 13(4):474-9. http://koomeshjournal.semums.ac.ir/article-1-1469-fa.html

[25] Drummond LR, Del Carlo RJ, Da Silva KA, Rodrigues AC, Soares PN, Gomes TN, et al. Enhanced femoral neck strength in response to weightlifting exercise training in maturing male rats. Int SportMed J. 2013; 14(3):155-167. https://journals.co.za/doi/epdf/10.10520/EJC142366

[26] Farsani ZH, Banitalebi E, Faramarzi M, Bigham-Sadegh A. Effects of different intensities of strength and endurance training on some osteometabolic miRNAs, Runx2 and PPARγ in bone marrow of old male wistar rats. Mol Biol Rep. 2019; 46(2):2513-21. [DOI:10.1007/s11033-019-04695-w] [PMID]

[27] Simpson KA, Mavros Y, Kay S, Meiklejohn J, de Vos N, Wang Y, Guo Q, et al. Graded resistance exercise and type 2 diabetes in older adults (the GREAT2DO study): Methods and baseline cohort characteristics of a randomized controlled trial. Trials. 2015; 16:512. [DOI:10.1186/s13063-015-1037-y] [PMID] [PMCID]

[28] Kelley GA, Kelley KS, Tran ZV. Resistance training and bone mineral density in women: A meta-analysis of controlled trials. Am J Phys Med Rehabil. 2001, 80(1):65-77. [DOI:10.1097/00002060-200101000-00017] [PMID]

[29] Aido MI. The influence of age and mechanical loading on bone structure and material properties [PhD dissertation]. Berlin: Technische Universität Berlin; 2015. https://pure.mpg.de/rest/items/item_2384739/component/file_2384743/content

[30] Cui S, Sun B, Yin X, Guo X, Chao D, Zhang C, et al. Time-course responses of circulating microRNAs to three resistance training protocols in healthy young men. Sci Rep. 2017; 7(1):2203. [DOI:10.1038/s41598-017-02294-y] [PMID] [PMCID]

[31] Boppart MD, De Lisio M, Zou K, Huntsman HD. Defining a role for non-satellite stem cells in the regulation of muscle repair following exercise. Front Physiol. 2013; 4:310. [DOI:10.3389/fphys.2013.00310] [PMID] [PMCID]

[32] Ogasawara R, Akimoto T, Umeno T, Sawada S, Hamaoka T, Fujita S. MicroRNA expression profiling in skeletal muscle reveals different regulatory patterns in high and low responders to resistance training. Physiol Genomics. 2016; 48(4):320-4. [DOI:10.1152/physiolgenomics.00124.2015] [PMID]

[33] Sawada S, Kon M, Wada S, Ushida T, Suzuki K, Akimoto T. Profiling of circulating microRNAs after a bout of acute resistance exercise in humans. PLoS One. 2013; 8(7):e70823. [DOI:10.1371/journal.pone.0070823] [PMID] [PMCID]

[34] Aoi W, Ichikawa H, Mune K, Tanimura Y, Mizushima K, Naito Y, et al. Muscle-enriched microRNA miR-486 decreases in circulation in response to exercise in young men. Front Physiol. 2013; 4:80. [DOI:10.3389/fphys.2013.00080] [PMID] [PMCID]

[35] Mayr M, Zampetaki A, Kiechl S. MicroRNA biomarkers for failing hearts? Eur Heart J. 2013; 34(36):2782-3. [PMID]

[36] Davidsen PK, Gallagher IJ, Hartman JW, Tarnopolsky MA, Dela F, Helge JW, et al. High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression. J Appl Physiol (1985). 2010; 110(2):309-17. [DOI:10.1152/japplphysiol.00901.2010] [PMID]

[37] Russell AP, Lamon S, Boon H, Wada S, Güller I, Brown EL, et al. Regulation of miRNAs in human skeletal muscle following acute endurance exercise and short-term endurance training. J Physiol. 2013; 591(18):4637-53. [DOI:10.1113/jphysiol.2013.255695] [PMID] [PMCID]

[38] Nielsen S, Scheele C, Yfanti C, Akerström T, Nielsen AR, Pedersen BK, et al. Muscle specific microRNAs are regulated by endurance exercise in human skeletal muscle. J Physiol. 2010; 588(Pt 20):4029-37. [DOI:10.1113/jphysiol.2010.189860] [PMID] [PMCID]

[39] Drummond MJ, McCarthy JJ, Fry CS, Esser KA, Rasmussen BB. Aging differentially affects human skeletal muscle microRNA expression at rest and after an anabolic stimulus of resistance exercise and essential amino acids. Am J Physiol Endocrinol Metab. 2008; 295(6):E1333-40. [DOI:10.1152/ajpendo.90562.2008] [PMID] [PMCID]

[40] Song J, Saeman MR, Baer LA, Cai AR, Wade CE, Wolf SE. Exercise altered the skeletal muscle microRNAs and gene expression profiles in burn rats with hindlimb unloading.J Burn Care Res. 2017; 38(1):11-9. [DOI:10.1097/BCR.0000000000000444] [PMID] [PMCID]

[41] D'Souza RF, Bjørnsen T, Zeng N, Aasen KMM, Raastad T, Cameron-Smith D, et al. MicroRNAs in muscle: Characterizing the powerlifter phenotype. Front Physiol. 2017; 8:383. [DOI:10.3389/fphys.2017.00383] [PMID] [PMCID]

[42] Trumbull A, Subramanian G, Yildirim-Ayan E. Mechanoresponsive musculoskeletal tissue differentiation of adipose-derived stem cells. Biomed Eng Online. 2016; 15:43. [DOI:10.1186/s12938-016-0150-9] [PMID] [PMCID]

[43] Turner CH, Takano Y, Owan I. Aging changes mechanical loading thresholds for bone formation in rats. J Bone Miner Res. 1995; 10(10):1544-9. [DOI:10.1002/jbmr.5650101016] [PMID]

[44] Razi H, Birkhold AI, Weinkamer R, Duda GN, Willie BM, Checa S. Aging leads to a dysregulation in mechanically driven bone formation and resorption. J Bone Miner Res. 2015; 30(10):1864-73. [DOI:10.1002/jbmr.2528] [PMID]

[45] Qin Y, Peng Y, Zhao W, Pan J, Ksiezak-Reding H, Cardozo C, et al. Myostatin inhibits osteoblastic differentiation by suppressing osteocyte-derived exosomal microRNA-218: A novel mechanism in muscle-bone communication. J Biol Chem. 2017; 292(26):11021-33. [DOI:10.1074/jbc.M116.770941] [PMID] [PMCID]

[46] Marinho R, Alcântara PSM, Ottoch JP, Seelaender M. Role of exosomal microRNAs and myomiRs in the development of cancer cachexia-associated muscle wasting. Front Nutr. 2018; 4:69. [PMID]

[47] Mitchelson KR, Qin WY. Roles of the canonical myomiRs miR-1,-133 and-206 in cell development and disease. World J Biol Chem. 2015; 6(3):162-208. [DOI:10.4331/wjbc.v6.i3.162] [PMID] [PMCID]