ORGINAL ARTICLE

The Effect of Elastic Resistance Bands Training on Vascular Aging Related Serum microRNA-146 Expression and Atherosclerosis Risk Factors in Elderly Women with Osteosarcopenic Obesity: A Randomized Clinical Trial

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Abstract

Objective: Vascular aging and osteosarcopenic obesity (OSO) refers to age-related endothelial cells and body composition disorders. The purpose of this study was to investigate the effect of 12 weeks resistance training with elastic bands on vascular aging-related serum miR-146 expression level and atherosclerosis risk factors in elderly women with OSO.

Materials and Methods: In the present single-blind randomized clinical trial, 48 elderly women with OSO (according to Dual energy X-ray absorptiometry (DEXA) method results), were divided randomly into two groups of control (n=22) and experimental (n=26). The experimental group performed resistance training with elastic bands for 12 weeks and three sessions per week for all major muscle groups. Statistical analyses were carried out using SPSS statistical software version 22.

Results: Between-group comparisons showed a significant decrease in serum miR-146 expression (*P*-value= 0.005) and total cholesterol (*P*-value= 0.034) in the experimental group compared to the control group. There was no significant difference in body weight (*P*-value= 0.440), BMI (*P*-value= 0.553), total fat (*P*-value= 0.093), BMC (*P*-value= 0.862), BMD (*P*-value= 0.564), Hs-CRP (*P*-value= 0.065), HDL-C (*P*-value= 0.515) and LDL-C (*P*-value= 0.889).

Conclusion: Resistance trainings decrease miR-146 expression in elderly women with OSO, which was associated with decreased LDL levels and increased HDL levels, though BMI, body fat percentage, total cholesterol and HS-CRP were not significantly different probably due to the type and intensity of the exercises that require further investigation.

Keywords: Exercise training, Elderly, Endothelial cells, microRNAs

Introduction

Based on the association between decreased muscle and skeletal mass and increased fat mass in the elderly, scientists have recently proposed the terms sarco-osteopenia, sarco-osteoporesis, sarcopenic obesity (SO), and osteosarcopenic obesity

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(OSO) (1-3). The OSO is caused by hormonal changes, decreased physical activity, decreased dietary protein or vitamin D deficiency as well as increased body fat percentage, and is associated with some catabolism conditions caused by chronic inflammation and osteoporosis (4).

Vascular aging (senescence) is a type of endothelial cell aging that is associated with age-related changes in the vessels, including atherosclerotic plaques, arterial stiffness, fibrosis and increased thickness of the medial layer of the vessels. The vascular aging can affect the severity of cardiovascular diseases, thus making it one of the most important risk factors and high mortality in cardiovascular diseases (5,6).

It is currently known that one of the microRNAs stimulating aging is miR-146, which increase vascular aging by reducing Sirtuin 1 (SIRT1) in endothelial cells (ECs), and stimulates vascular smooth muscle cells (VSMC) apoptosis by activating the nuclear factor kappa B (NF-κ B) signaling pathway (7,8).

Traditional resistance training or free weights exercises put on great stress on the musculoskeletal system and joints and are unsafe particularly for the elderly people (9-11). Elastic bands are widely used today in rehabilitation of special group because of their cost-effectiveness, easy accessibility, impact on body composition, physical function and physiological adaptation, and ease of performing a range of upper and lower body exercises in any extraverted and introverted location (12,13).

Several vascular aging-related endothelial proteins are affected by aerobic exercise (14).

Both aerobic exercise and resistance training improve endothelial function. The aerobic exercise is associated with lower levels of stiffness in the central arteries, indicating that regular exercise can reduce or prevent aging in arterial stiffness (15). In contrast, the effect of resistance training is not as well-known as aerobic exercise, although current data suggest a role in ameliorating endothelial dysfunction (16). The resistance training is also associated with lower levels of arterial adaptation. However, chronic resistance training has been shown to improve vascular function and reduce the risk of hypertension (16,17). In addition, there is an inverse correlation between muscular strength and aortic stiffness, suggesting that improving muscle strength may also be beneficial for vascular health. Williams et al. (2013) showed that resistance training can lead to improved vascular function in older women (18).

The number of studies on circulating miRNAs affected by both acute and chronic exercises has increased over the last few years (19,20). For example, Baggish et al. reported rapid upregulation of miR-146a in response to acute exercise, and showed a return of miRNA plasma levels after one-hour of resting (21). Another study found a decrease in miR-146a levels and suggested that miRNA levels change in response to acute resistance exercise, and miRNAs play an important role in adaptation induced by resistance training (21). Few studies have documented altered circulating miRNAs after prolonged exercise protocols. Baggish et al. reported increased baseline miR-146a levels after three months of moderate-intensity sailing training (22).

Due to the complexity in vascular adaptations to resistance type exercise training, specific vascular aging markers to exercise training can serve as markers for control changes of risk factors related to vascular aging.

So, this trial was to study the effects of elastic resistance training on biomarkers of vascular aging syndrome. In the current study, we hypothesized that the resistance training using elastic bands may improve age-related decline in endothelial function. These hypotheses were tested by examining vascular aging-related serum miR-146 expression level and atherosclerosis risk factors in elderly OSO women following 12-week resistance training with elastic bands. This study may provide new evidence for further research on the critical role of resistance exercise as an effective lifestyle intervention to maintain

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vascular homeostasis in the elderly population. Therefore, the present study aimed to investigate the responses of miR-146 and some atherosclerosis risk factors (HDL, LDL, hs-CRP and cholesterol) following resistance training in elderly women with OSO.

Materials and Methods

This randomized clinical trial (RCT) was performed based on the CONSORT statement for randomized trials of non-pharmacologic treatments (23,24). Sample size was calculated 48 people according to: 1- Statistical method; 2- Existence of two groups; 3- Type I error of 5%; 4- Type II error of 20%; 5- Test power of 80%; and 6- Effect size of 0.20 using G * Power software (version 3.1.9.2). The final sample size was estimated to be 63 people by considering 20% dropout. All eligible patients were selected by the same physician. In this study, eligible patients were selected using dual-energy X-ray absorptiometry (DEXA), age range of 60-80 years, body fat percentage of >32%, body mass index (BMI) of >30 kg/m^2, -2.5≤ T-score≤-1 from L1-L4 or whole femur or femoral head, and 10-m walking speed (10 MWT) of ≤1 meter per second squared (25,26). All participants were also evaluated based on other inclusion criteria, including no chronic disease such as hypertension, thyroid or kidney problems, cancer, diabetes or very severe osteoporosis (T-score< -2.5). No hormone therapy, irregular exercise for more than 30 minutes during the past six months, no dietary supplements for the past 3 months were considered as well by physician. Exclusion criteria were parallel exercise, following a weight loss diet of more than 5kg in the last three months, hormone therapy, or taking any medication that affects bone density, adipose tissue, or the hormonal system (Figure 1).

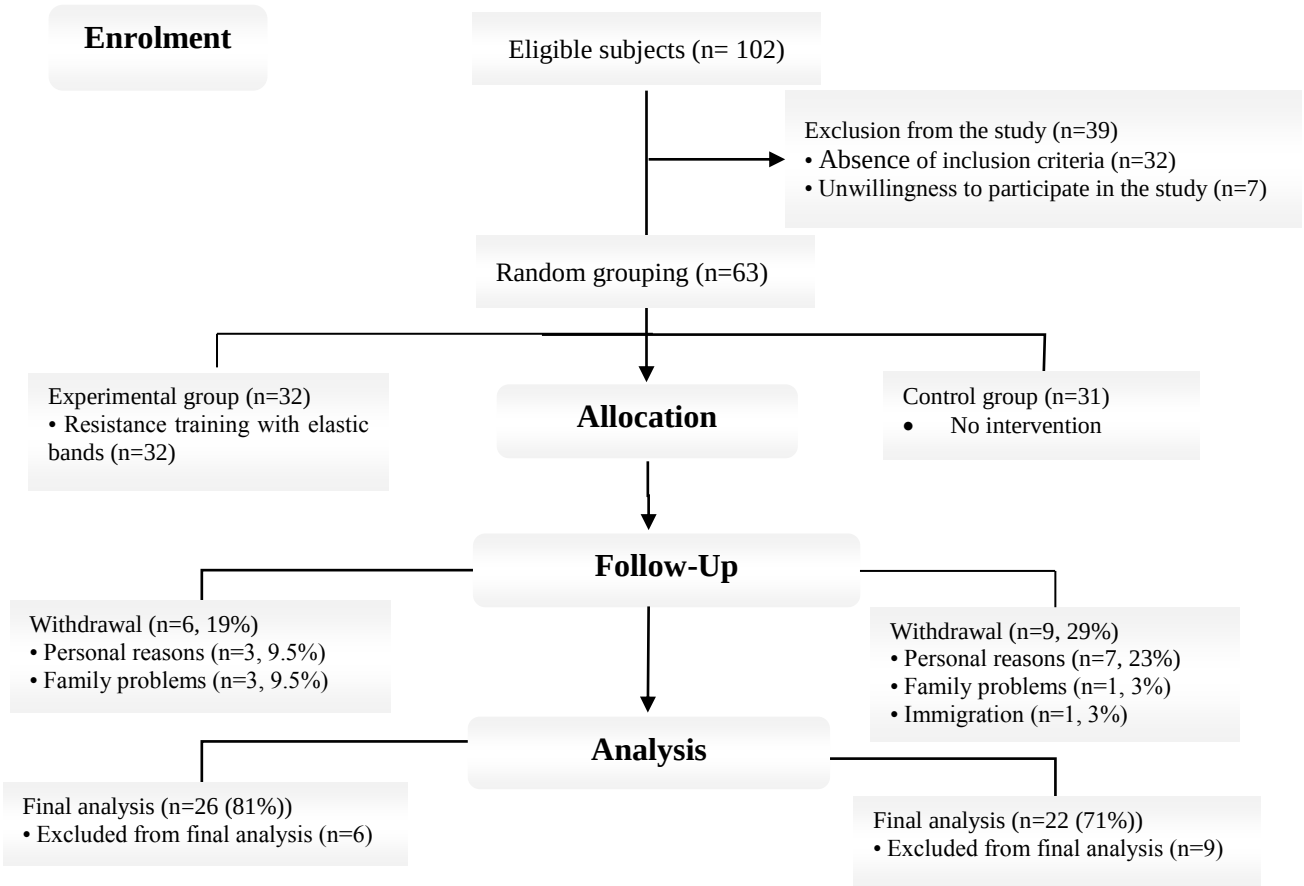

Figure 1. CONSORT flow diagram

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Informed written consent was obtained from all patients after initial evaluation and randomization (27). This randomization was also performed by an external researcher using a random replacement block size of block 4, not involved in experimental process or training programs. Participants were stratified according to two cut-offs for each stratification of age (60–70 or 70–85 yrs) and OSO-z score (−3 to 0 or 0 to +3). This allocation is mostly hidden by those in charge of designing the exercise training protocol or supervising the control group until the beginning of the sports training course. Neither participants nor researchers were blinded by the nature of the intervention. Besides; exercise trainers, not involved in data collection, did the exercise session program and monitored the individuals in the control group. Participants in the control group also did not receive any dietary intervention or changes in their normal diet or physical activity during the study period. They received phone calls or face-to-face interviews once a week to make sure the study did not alter their physical activity and diet. In these weekly visits; health problems, functional problems, as well as drug use were recorded by a trained researcher. At the same time, the researchers strengthened their commitment to regular diet and exercise habits.

Blood sampling and DEXA method were performed for eligible subjects after measuring the initial functional tests. First, for 2 weeks and three sessions of 60 to 90 minutes per week, the resistance training was performed by yellow elastic bands to familiarize with the exercise tools, training environment and correction of patient movements. Then, the exercise program started for 12 weeks. After 48 hours of the last training session, blood sampling, DEXA METHOD, anthropometric, functional and laboratory measurements were performed at the time and conditions of the initial tests by the same researcher and laboratory expert. In addition, the OSO conditions of patients were evaluated using the following equation.

(Muscular strength Z-core) + (SMM Z-score) + (−1 × body fat Z-score) + (BMD Z-score)/4.

The patients were trained about the exercise method during two sessions prior to the start of exercise protocols. In addition, the patients were trained to control exercise intensity using the targeted number of repetitions (TNRs) and the OMNI-resistance exercise scale (OMNI-RES) in the first two sessions (28,29). In the resistance exercises with elastic bands, the patients can easily adjust the resistance intensity by increasing or decreasing the arm distance. They were asked to choose a suitable elastic band that allowed them to perform 20-RM (29).

In general, resistance training with elastic bands (Thera Band®, Hygienic Co., Akron, OH, USA) was designed and accomplished to train all the major muscle groups (exercise volume and intensity were constantly increased) three times a week under researcher control and supervision. The patients were divided into four groups of 6-8 each to facilitate control and monitoring and to increase the accuracy and correctness of the movements. Each group participated in a training session at a specified time under the direct and full supervision of the researcher. The exercises were performed according to the guidelines of the American College of Sports Medicine (ACSM) recommended for elderly resistance training. Each training session began with a 10-minute warm up, then resistance training with elastic band (35-40 minutes) was performed in a controlled and slow manner for each of the six muscle groups (legs, back, abdomen, chest, shoulder and arm). The training session ended with 5-minute cool down. In order to adhere to the principle of overload after every 2 weeks of training, the intensity of exercise was increased by using elastic band discoloration. Accordingly, they were changed from yellow to red, blue, green, black and silver, respectively. In addition, training volume increased with increasing number of sets from one to two sets and progression based on individual recovery (the patient was able to

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perform two more repetitions in the second set, and reported resistance of less than 7 (0: very easy to 10: very difficult) for active muscle based on the OMNI scale (29) and changed the color of elastic band). It should be noted that all training programs were performed daily between 8:00 and 12:00.

The blood samples were taken 12 h before the fasting test; 5 ml blood was obtained from the brachial vein. In addition, 5 ml of blood was kept at room temperature for 10 minutes to make a clot, and then centrifuged to extract serum to measure the research variables. High-sensitivity C-reactive protein (hs-CRP), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and total serum cholesterol were measured in mg/ml by ELISA technique using the kit (Pars Azmun Company). The miR-146 expression levels were measured in blood serum samples by Real Time RT-PCR, and the U6 gene was selected as the control gene. For statistical analysis, mean and standard deviation were used as descriptive statistics. Kolmogorov-SmiRnov test was used for data normality. Independent sample t-test was performed to compare groups in the baseline, as well as for the gene expression comparisons. Two-way ANOVA (group*time) was applied for between-group comparisons. Effect size (ES) was calculated using Partial Eta squared test. All tests were carried out at the significance level of 0.05 using SPSS 22 software.

Ethical considerations

The trial was registered at the Iranian registry of clinical trials (http://www.irct.ir) with the IRCT20180627040260N1; https://www.irct.ir/trial/32463. This study was approved by Iranian Ethics Committee of Sport Sciences Research Center. (IR.SSRC.REC.1398.012). All the study participants also provided written informed consent.

Results

Nine (29%) participants in control group and six (19%) of participants in experimental group did not complete the post-test measurements. There was an 85% adherence rate in training sessions in the training group. No significant adverse events were reported by participant during training period.

The result of independent-sample T-test showed that there were no significant differences in baseline characteristics between study groups (Table 1).

Result of independent sample T-test for between-group comparisons of gene expression data showed a significant decrease in serum miR-146 expression (*P*-value= 0.005). Also, the result of two-way ANOVA test for between group comparisons showed significant decreased in total cholesterol (*P*-value= 0.034) in the experimental group compared to the control group. There was no significant difference in body weight (*P*-value= 0.440), BMI (*P*-value= 0.553), total fat (*P*-value= 0.093), BMC (*P*-value= 0.862), BMD (*P*-value= 0.564), Hs-CRP (*P*-value= 0.065), HDL-C (*P*-value= 0.515) and LDL-C (*P*-value= 0.889) (Table 2). There was a significant correlation between hs-CRP with BMI and body fat percentage (r= 0.50 and *P*-value= 0.003, r= 0.39 and *P*-value= 0.011, respectively) (Figure 2).

Discussion

The 12-week resistance training with elastic bands decreased LDL levels and significantly increased HDL levels in the experimental group compared to the control group, while no significant difference was found in body weight, BMI, body fat percentage, cholesterol and HS-CRP.

Few studies have investigated the effect of resistance training on hs-CRP levels in comparison with the aerobic exercise, with most of these studies reporting a decrease, increase or no change. Kout et al. observed no significant changes in hs-CRP levels after 10 months of resistance training among elderly bodybuilders with moderate intensity, which is consistent with the results of the present study (30).

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Kuhi et al. examined the effect of resistance training on hs-CRP in obese men with BMI greater than 30 kg/m2. The protocol of resistance training was 12-week exercise with

Table 1. Baseline characteristics of participants in both groups

Variable	Group	Baseline	*P*-value
Age (years)	Control (n=31) Experimental (n=32)	64.05 (±3.35) 64.11 (±3.81)	0.947
Height (cm)	Control (n=31) Experimental (n=32)	155.77 (±4.14) 155.59 (±4.38)	0.812
Weight (kg)	Control (n=31) Experimental (n=32)	78.73 (±7.52) 81.81 (±8.03)	0.268
Body mass index (BMI) (kg/m^2)	Control (n=31) Experimental (n=32)	32.53 (±2.01) 33.72 (±3.15)	0.451
Body fat %	Control (n=31) Experimental (n=32)	43.60 (±2.66) 46.29 (±3.42)	0.067
Bone mass content (BMC) (gr)	Control (n=31) Experimental (n=32)	2.13 (±0.50) 2.24 (±0.38)	0.381
Bone mass density (BMD) (gr/cm^2)	Control (n=31) Experimental (n=32)	1.005 (±0.450) 0.929 (±0.245)	0.374
Muscle quality (MQ) (W)	Control (n=31) Experimental (n=32)	578.42 (±100.46) 563.90 (±101.92)	0.453
Gait speed (10MWT) (m/s)	Control (n=31) Experimental (n=32)	0.883 (±0.62) 0.864 (±0.61)	0.386
Six minutes walking test (6MWT) (m)	Control (n=31) Experimental (n=32)	306.57 (±58.86) 302.93 (±42.45)	0.184
30s chair-stand test (Reps)	Control (n=31) Experimental (n=32)	7.64 (±1.28) 8.71 (±1.44)	0.364
Timed Up and Go (TUG) Test (s)	Control (n=31) Experimental (n=32)	15.91 (±4.47) 13.49 (±2.95)	0.652
Hand grip strength (kg)	Control (n=31) Experimental (n=32)	20.48 (±4.12) 20.54 (±3.37)	0.247

Table 2. Effects of 12 weeks resistance training

Variable	Group	Time of measurements		Δ%	*P*-value Between group	*p*-value within group
		pre	post			
Age (years)	Control (n=31) Experimental (n=32)	64.05 (±3.35) 64.11 (±3.81)			0.947	
Height (cm)	Control (n=31) Experimental (n=32)	155.77 (±4.14) 155.59 (±4.38)	155.08 (±4.59) 156.15 (±4.89)	-0.44 0.36	0.889	0.001
Weight (kg)	Control (n=31) Experimental (n=32)	78.73 (±7.52) 81.81 (±8.03)	81.66 (±10.09) 81.87 (±9.82)	3.72 0.07	0.440	0.007
Body mass index (BMI) (kg/m2)	Control (n=31) Experimental (n=32)	32.53 (±2.01) 33.72 (±3.15)	33.33 (±4.05) 33.65 (±3.67)	0.73 -0.06	0.553	0.004
Total fat %	Control (n=31) Experimental (n=32)	43.60 (±2.66) 46.29 (±3.42)	47.60 (±2.65) 47.35 (±3.86)	9.17 2.29	0.093	0.030
Bone mass content (BMC) (g)	Control (n=31) Experimental (n=32)	2.13 (±0.50) 2.24 (±0.38)	2.11 (±0.53) 2.26 (±0.44)	-0.94 0.89	0.862	0.001
Bone mass density (BMD) (g/cm2)	Control (n=31) Experimental (n=32)	1.005 (±0.450) 0.929 (±0.245)	0.947 (±0.274) 0.945 (±0.271)	-5.77 1.72	0.564	0.004
High-sensitivity C-reactive protein (hs-CRP) (mg/ml)	Control (n=31) Experimental (n=32)	3.874 (±1.596) 3.015 (±1.672)	3.377 (±1.804) 3.020 (±1.779)	-12.83 0.166	0.065	0.042
High-density lipoprotein (HDL) cholesterol (HDL-C) (mg/ml)	Control (n=31) Experimental (n=32)	47.082 (±6.865) 47.895 (±6.267)	50.100 (±8.416) 41.94 (±8.532)	6.41 12.42	0.515	0.007
Low-density lipoprotein (LDL) cholesterol (mg/ml)	Control (n=31) Experimental (n=32)	90.536±22.682 94.972 (±24.84)	98.71±25.33 85.009 (±23.12)	9.03 10.49	0.889	0.001
Total Cholesterol (mg/ml)	Control (n=31) Experimental (n=32)	167.727 (±35.22) 181.227 (±65.95)	177.64 (±42.23) 167.68 (±40.74)	5.91 -7.47	0.034	0.071

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an intensity of 60-80% of one-repetition maximum, and 8 to 10 repetitions at 10 stations. The serum HS-CRP levels in the intervention group were significantly decreased after the study exercises compared to the control group (31). Possible mechanisms for altering hs-CRP levels may be due to decreased body fat mass and increased anti-inflammatory cytokines (30-32). According to the results of this study, no significant changes in h-CRP are likely to be associated with no changes in body fat mass. In addition, no significant change in body fat percentage and blood cholesterol was associated with decreased and increased levels of LDL and HDL, respectively. However, most studies reported a decrease in fat, cholesterol, LDL, and HDL levels after resistance training in obese older people (33,34), probably due to the measurement methods and tools (using the DEXA method in fat estimation) or the type and intensity of exercise selected, including the use of a moderate-intensity elastic band.

The study results showed a significant decrease in serum miR-146 expression in the experimental group compared to the control group. The role of miRNAs in the regulation of inflammation, cardiovascular health and cardiovascular disease (CVD) is currently well established and a reverse association has been reported between miR-34a, miR-126, miR-146a, miR-150 and miR-181b with severe vascular inflammation, endothelial dysfunction and atherogenesis (35).

Many studies have pointed to the role of miR-146 in the cellular processes of obesity and its increased expression in adipocytes of obese and cardiovascular patients. Shi et al. (2018) reported an increase in serum miR-146 expression along with an increase in inflammatory markers in elderly patients with acute myocardial infarction (AMI) compared to healthy controls. They found a significant reverse correlation between serum miR-146 expression and inflammatory markers such as TNF-α and HS-CRP. They suggested that the effect of miR-146 on the inhibition of inflammatory markers should be investigated.

Ultima et al. (2018) examined serum miRNA levels of 12 healthy men. The training protocol was a resistance training session (leg press and knee press), five sets of 10 repetitions at 70% of maximal intensity, with 1-minute rest between each set. Three days after training, they observed a decrease in the miR-146 levels (21). Nielsen et al. (2014) reported that 60-minute acute cycle ergometer exercise with 65% of maximal intensity resulted in an increase in serum miR-146 expression, whereas long-term exercise led to a decrease in serum miR-146 expression (36). Decreased expression of cardiac miR-208a in healthy and obese Wistar rats could result in overregulation of thyroid hormone receptor-

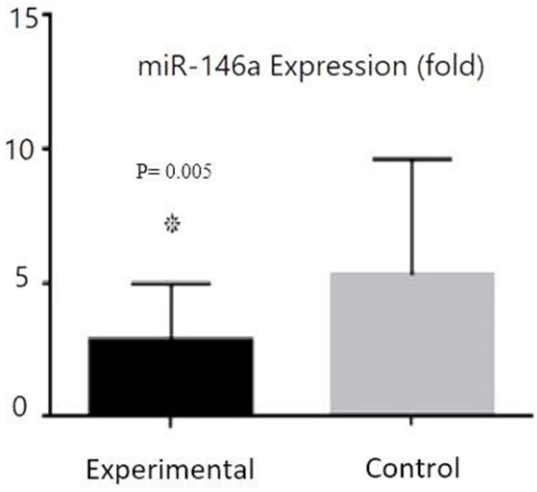

Figure 2. miR-146a expression

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associated protein-1 (THRAP-1), Purβ and Sox6, and improved balance between cardiac α- and β myosin heavy chain (α-β MHC) genes (37).

There were some study limitations in this trial. We could not monitor the effect of social parameters on outcomes. In addition, short-term exercise training period is the second limitation of this study that is a reason for non-significant effect of this exercise training protocol on them. Small sample size is third limitation of this study.

Conclusions

Overall, the results of this trial showed significant effect only in gene expression of miR-146a between two groups in women with OSO. Resistance type exercise training with elastic band caused non-significant improvements in total serum vascular aging biomarkers. This trial protocol could apply for decision-making regarding the optimal protocol for elderly women with OSO. More research is thus suggested to compare machine- and elastic band- resistance training protocols at different intensities and volumes.

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Conflict of Interest

Ayoub Hashemi, Rahman Soori, Ebrahim Banitalebi and Siroos Choobineh declare that they have no conflict of interest.

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