Research Article

Influence of concurrent and functional training on miR-1/miR-126 gene expression and cardiovascular function in postmenopausal women

Majid Kashef[1], Fereshte Shahidi[2], Hadi Samadi[3], Leyla Golroo[4*]

Abstract

It is becoming more important to understand the physiological processes that contribute to the emergence of risk factors associated with menopause, and to discover strategies for preventing and reversing them. The current research aimed to determine the impact of functional and concurrent training on miR-126/miR-1 gene expression, and cardiovascular function in middle-aged postmenopausal women. In this study, 24 women between the ages of 50 and 60 years old with a sedentary life style participated. The subjects were randomly divided into the control group, concurrent training group, and functional training group. The Control received no intervention, and the experimental groups performed selected training protocols for 12 weeks. Doppler ultrasound graphs were utilized after the interventions to evaluate the cardiovascular function. Also, 48 hours after experiment, the blood collection was taken to assess miR-126/miR-1 gene expression. To compare the variables, one-way ANOVA test followed by Tukey's post hoc test was used. The significance level was set at $p < 0.05$. The results showed that the training programs led to significant enhancements in FLOW-mediated dilation, and PSV (peak systolic velocity). Moreover, there was a significant decrease in the vascular stiffness in the participants after undergoing the training. Additionally, miR-1 and miR-126 gene expression were found to be increased in response to both training interventions. These findings suggest that concurrent and functional training may be an effective approach for reducing the risk of age-related diseases in menopausal women through promoting cardiovascular functions and miR-126/miR-1 gene expression elevation.

Key Words: **Menopause, MicroRNAs, Stiffness index, PSV**

1. Professor at Exercise Physiology, Exercise Physiology Department, Sport Sciences Faculty, Shahid Rajaee Teacher Training University, Tehran, Iran. 2. Associate Professor at Exercise Physiology, Exercise Physiology Department, Sport Sciences Faculty, Shahid Rajaee Teacher Training University, Tehran, Iran. 3. Assistant Professor at Exercise Physiology, Exercise Physiology Department, Sport Sciences Faculty, Shahid Rajaee Teacher Training University, Tehran, Iran. 4. Ph.D. candidate at Exercise Physiology, Exercise Physiology Department, Sport Sciences Faculty, Shahid Rajaee Teacher Training University, Tehran, Iran

*Author for correspondence: leylagolroo@sru.ac.ir

M K: 0000-0001-8551-3371; F Sh: 0000-0001-6593-4435; H S: 0000-0002-0618-2437; L G: 0000-0005-7099-1123

Received 28 October 2023; Accepted 31 December 2023

Introduction

With the increasing number of elderly women and their growing life expectancy, there are mounting health concerns regarding changes that occur during the post-menopausal period (Lunenfeld and Stratton 2013). Menopause is a natural physiological event that occurs when a woman's ovarian follicle function declines, leading to reduced production of ovarian hormones and the permanent cessation of menstrual cycles (Montenont, Rondina, and Campbell 2019). From a medical perspective, menopause is defined as the absence of menstruation for 12 consecutive months (McKinlay, Brambilla, and Posner 1992). It is estimated that the population of menopausal women will reach 1.2 billion by the year 2030, with an annual increase of 47 million new cases (Tan and Dayu 2022). It has been linked to multiple health risks such as diabetes, skeletal muscle wasting (sarcopenia), loss of bone mineral mass (osteopenia), changes in body composition, lipid profile, fat deposition, increased inflammatory markers, and coronary artery diseases (Ko and Kim 2020). Worldwide, heart diseases are the main causes of death among post-menopausal women (Madika et al. 2019). This finding has led to the theory that menopause plays a role in the increased incidence of coronary diseases (Xiang et al. 2021). The traditional risk factors for cardiovascular disease include a sedentary lifestyle, older age, smoking, poor diet, hypertension, diabetes mellitus, dyslipidemia, high BMI, and family history (Bays et al. 2021).

There is an increasing need to comprehend the physiological mechanisms involved in the development of menopause-related risk factors and to devise strategies for their prevention and reversal. Hormone replacement therapy, dietary modifications, and physical exercise are recommended as important components of treatment for menopause-related risk factors (Leite et al. 2010). Hormone replacement therapy is commonly prescribed to alleviate the negative effects of menopause, but it is associated with several risks in humans, notably an increased incidence of certain types of cancer (Biglia et al. 2007). As a result, various exercise modalities are recom-

-mended to help manage menopause-related symptoms. These modalities include continuous and intermittent exercises like walking and running, as well as flexibility exercises and resistance training (Leite et al. 2010). Resistance training has become increasingly popular due to its effectiveness in treating several conditions related to menopause, including osteopenia, sarcopenia, diabetes, cardiovascular disease, among others (Leite et al. 2010). The loss of muscle strength due to aging can impair the ability to perform daily activities and increase the risk of disability (Kalyani, Corriere, and Ferrucci 2014). Numerous studies have demonstrated that progressive resistance strength training enhances muscle strength in older adults, and is therefore recommended as a means of preventing or reducing late-life disability (Liu and Latham 2011). In recent years, functional and concurrent training have garnered attention as new exercise modalities. It has been known that functional training can be an effective approach for improving activities of daily living (ADL) performance in older adults (Liu et al. 2014). This method focuses on training muscles in coordinated, multiplanar movement patterns that involve multiple joints, dynamic tasks, and changes in the base of support (Stenger 2018). By improving function, functional training can be any type of exercise that is performed with the goal of enhancing a specific movement or activity (Hibbs et al. 2008). Also, concurrent training refers to the incorporation of both resistance training, which promotes strength, hypertrophy, and power, and aerobic exercise, which enhances endurance, into a single program (Hartono et al. 2022). Resistance training focuses on training skeletal muscle in short-duration activities, with maximal or near-maximal force levels (Wilson et al. 2012). On the other hand, endurance training necessitates individuals to exert relatively low force outputs over extended periods (Wilson et al. 2012). Consequently, the adaptations for resistance and endurance exercises are considerably different and assessing their physiological responses can reveal those differences.

Numerous biomarkers are available to monitor physiological responses to different types of exercise. Recent research has highlighted the potential of miRNAs present in circulation as a new biomarker used in the diagnosis of various cardiovascular diseases. Micro RNAs (miRNAs) are small RNA molecules that do not code for proteins but play a crucial role in regulating gene expression after transcription by inhibiting or activating mRNA (Gupta, Bang, and Thum 2010). In the human body, miRNAs are vital regulators involved in numerous physiological processes such as proliferation, differentiation, development, and apoptosis. The transportation of miRNAs into the blood occurs through various mechanisms, including fusion proteins (like HDL), exosomes, and microvesicles (Duarte, Palmeira, and Rolo 2014). The transporting of miRNAs with these complexes enables them to circulate in the bloodstream and remain stable, preventing degradation, allowing them to act on target cells throughout the body (Dilsiz 2020). Studies have shown that the baseline miRNA profile correlates significantly with myocardial infarction (Ai et al. 2010). Additionally, miRNAs have been identified as potential predictors for various disorders. Studies have shown that levels of miR-126 and miR-1 are significantly associated with the prevention of atherosclerosis and myocardial hypertrophy respectively (Kura et al. 2019). Specifically, miR-126 is expressed in endothelial cells and plays a role in regulating endothelial cell migration, cytoskeletal reorganization, capillary network stability, cell survival, and apoptosis (Soci et al. 2017). Thus, miR-126 plays a crucial role in atherosclerosis by mobilizing progenitor cells to the site of apoptosis, leading to endothelial cell proliferation and a decrease in atherosclerosis (Carresi et al. 2021). Additionally, miR-1 is expressed by cardiac fibroblasts (Wang et al. 2016), which recruits progenitor cells from the bone marrow to the peripheral circulation and helps protect the heart against fibrosis (Aliyeva et al. 2023). Studies have reported that levels of miR-1 are reduced or inhibited in individuals with heart failure, making it a significant biomarker in cardiovascular diseases (Jian et al. 2021).

Therefore, the focus of the current research is to determine the impact of functional and concurrent training on miR-126 and miR-1 gene expression in blood, and assessing their ability to enhance cardiovascular function in postmenopausal women.

Materials and Methods

Study characteristics

This was an experimental and controlled study. The procedures performed in this study were in accordance with the Ethical Standards of the Institutional and/or National Research Committee (IR.SSRI.REC.1401.177).

Sample selection

The study recruited female volunteers between the ages of 50 and 60 years' old who had not participated in any other structured training program within the past six months. A meeting was held to provide an overview of the project logistics, distribute the schedule of activities, and obtain written consent from the participants. Anthropometric assessments and blood collections were scheduled on specific days, at specific times, and at specific locations.

Design of the experiment

First, the entire sample was submitted to anthropometric assessment. Then, they were randomly divided into the control group, concurrent training group, and functional training group. The Control received no intervention, and the experimental groups performed selected training protocols for 12 weeks. Also, 48 hours after experiment, all participants returned for a blood collection and Doppler ultrasonography. Prior to measurements

of selected variables, participants were instructed to not eat 2-3 hours before, not drink alcohol, and not perform any physical exercise for 24 hours prior to assessment

Functional training protocol

The functional training program consisted of 11 exercise stations arranged in a circuit format, which the participants completed three times with a 30-second pause between each station. After completing the exercise stations, the participants performed a 27- to 30-minute walk on a treadmill, which depended on the overload performed during the exercise. The functional exercises included sit-ups, arm curls, lateral raises, seated rows, knee flexions, crucifixes, handle triceps, and squats, which were performed using elastic bands and free weights. The agility drills were conducted using movement between cones, agility ladders were used for coordination and Bosu ball was used for balance exercises. The FT program was divided into two phases: phase 1, which lasted from the 1st to the 6th week, involved 40 seconds of exercise and a 30-second pause between exercises. Phase 2, which lasted from the 7th to the 12th week, involved 50 seconds of exercise and a 30-second pause between exercises (Rossi et al. 2017).

Concurrent training protocol

The concurrent training protocol involved various resistance exercises, including leg press, leg extension, leg curl, bench press, seated row, arm curl, triceps extension, side elevation with dumbbells, and abdominal exercises. After the resistance exercises, the participants walked on a treadmill for about 30 minutes. The resistance training program was divided into two progressive phases. Phase 1, which lasted from the 1st to the 6th week, involved 12-15 repetitions, three sets per exercise, and a 60-second rest between sets. Phase 2, which lasted from the 7th to the 12th week, involved 10-12 repetitions, three sets per exercise, and a 60-second rest between sets. The training load was adjusted every four training weeks based on maximum repetitions (Rossi et al. 2017).

Doppler ultrasonography assessment

To perform the Doppler ultrasound graphs, the patient was comfortably positioned on an examination table with the area to be examined exposed. A gel was applied to the skin over the area of interest to enhance the transmission of ultrasound waves and improve image quality. Then, the ultrasound transducer, which both emitted and received the ultrasound waves, was placed on the skin over the area of interest. The ultrasound machine (The SuperSonic® MACH™ 30 ultrasound) was turned on and set to the Doppler mode, which allowed the machine to detect the movement of blood through the blood vessels. After the exam, the gel was wiped off the skin, and the patient was able to resume normal activities. A trained physician analyzed the images and measurements obtained during the exam to evaluate the patient's cardiovascular function.

Flow-mediated dilation (FMD) assessment

The subjects rested for 15 minutes in a supine position before the test. A blood pressure cuff placed on the non-dominant arm and inflated to suprasystolic pressure for 5 minutes. The cuff then rapidly deflated, and blood flow measured using a high-resolution ultrasound of the brachial artery. The diameter of the brachial artery measured at rest, during cuff inflation, and during peak hyperemia, which occurs 60-90 seconds after cuff deflation. FLOW-mediated dilation calculated as the percentage increase in diameter during hyperemia compared to baseline (Thijssen et al. 2011).

The vascular stiffness index

The protocol for assessing vascular stiffness using the Stiffness Index (SI) was based on the following steps:

1. Measuring the systolic and diastolic blood pressures using a sphygmomanometer.

2. Measuring the diameter of the brachial artery at both systolic and diastolic phases using ultrasound imaging.

3. Calculating the ratio of the diameters at systolic and diastolic phases

4. Using the SI formula to calculate the stiffness index

Blood collection

Trained pharmaceutical professionals collected blood samples from the study participants in accordance with recommended biosafety standards. The samples were taken at 48 hours after the last day of the experiment. All participants were directed to the laboratory at 6 am after fasting for 8-12 hours. Approximately 14 mL of blood was drawn from each subject using a vacuum tube and transferred to a 4 mL tube containing EDTA as an anticoagulant. The gene expression of miR-1 and miR-126 in Platelets was analyzed from this sample.

Analysis of gene expression

Total RNA Extraction and cDNA and miR-1 and miR-126 Preparation

Table 1. Sequences of primers.

Gene	Primer sequence 5'-3'
miR-1	F: TAAAGTGGGGACAGCAAAATGC R: AGCACAAGGTAGAGAAGGTAGAG
miR-126	F: GCGGCGGTCGTACCGTGAGTAA R: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGAT
β-Actin	F: GAGACCTTCAACACCCCAGCC R: CCGTCAGGCAGCTCATAGCTC

Blood samples were collected in EDTA tubes and centrifuged at 1500 g for 15 minutes to separate the plasma and the buffy coat from the red blood cells. Two thirds of the platelet-rich plasma were transferred to a new tube and mixed with PBS. The tube was then centrifuged at 800 g for 10 minutes to pellet the platelets. The supernatant was discarded and the platelet pellet was resuspended in Tyrode's buffer. The total RNA was resuspended in RNase-free water and quantified using a GeneQuant 100 spectrophotometer after that, cDNA was synthesized from 200 µg of total RNA, using the RevertAid First Strand cDNA Synthesis Kit, according to the manufacturer's recommendations. In short, approximately 200 µg of total RNA was reverse transcribed in a final volume of 20 µL of reaction. The resulting cDNA was stored at −30°C until real-time PCR analysis. β-Actin mRNA was used as the endogenous reference gene. Relative expression was calculated using the 2-ΔCt method. The real-time PCR was performed using the QuantStudio™ 6 Flex Real-Time PCR System with miRNA-specific stem-loop primers and TaqMan probes. The primers and probes were designed using the Primer Premier 6.1 Program (Premier Biosoft) and synthesized by a commercial company. The following primers sequences were synthesized (table 1).

Statistical analysis

The statistical analysis was performed using the software SPSS software. First, the Shapiro–Wilk normality test was performed, considering parametric tests ($p > 0.05$). The descriptive analysis of the variables, between the groups, was presented as mean an-

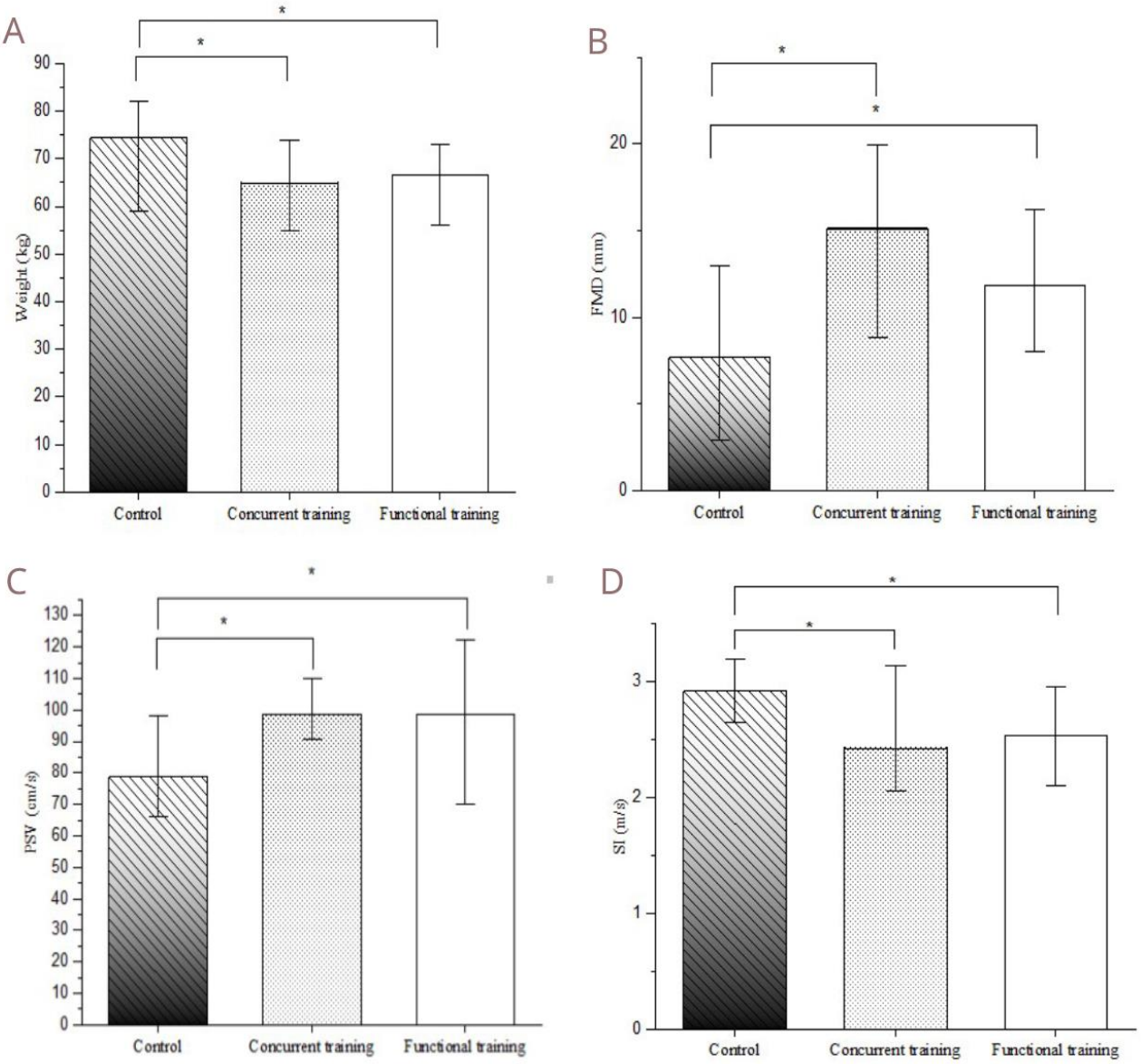

Figure 1. Descriptive characteristics and clinical parameters of participants. *Shows significant difference compared to CO at $p < 0.05$.

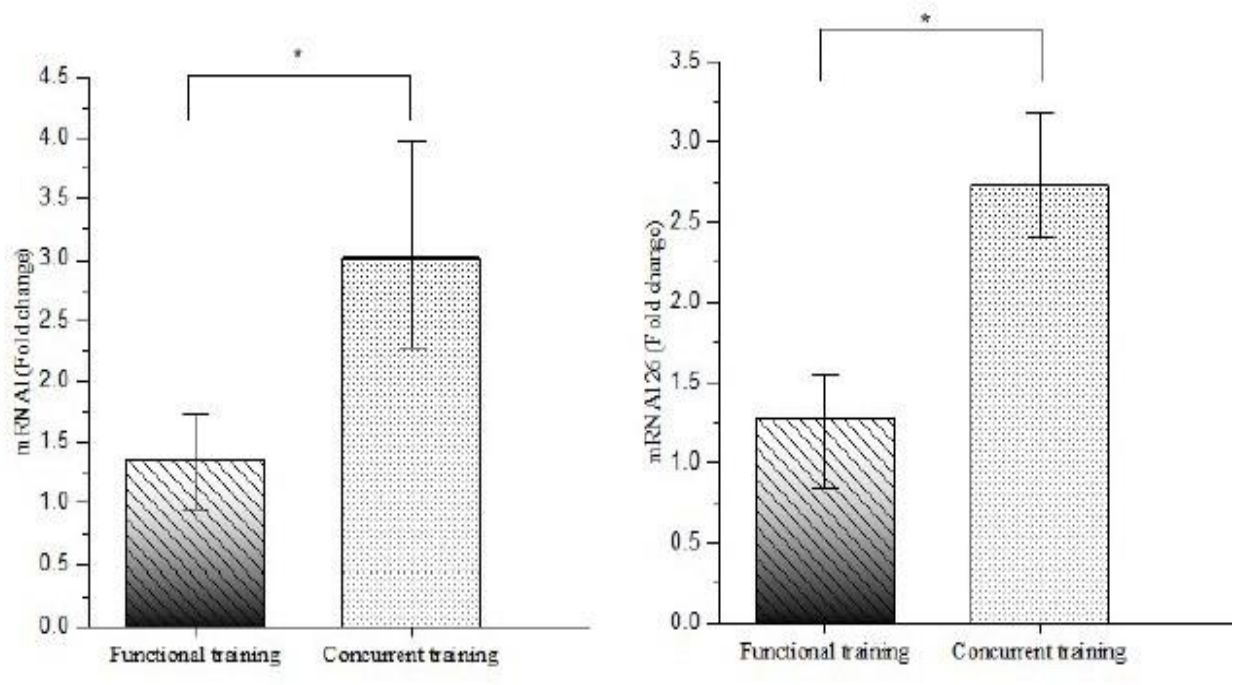

Figure 2. Fold change of miR-1 and miR-126 genes. *Shows significant difference at $p < 0.05$.

-d standard deviation, to compare the variables, one-way ANOVA test—paired for repeated measures and followed by Tukey's post hoc test—was performed. The significance level was set at $p < 0.05$.

Results

The study utilized One Way Analysis of Variance method and Least Significant Difference post-hoc test to analyze the variable changes. The findings revealed a significant reduction of weight in CT (P = .020) and FT (P = .044) groups (Figure1A). Statistical analysis also indicated that there was no significant difference in weight reduction between CT and FT groups (P = .712). Brachial artery FMD increased in the CT (P = .001) and FT (P = .032) groups compared to the CO group after 12 weeks (Figure1B). The increase in FMD was not significantly different between CT and FT groups (P = .082).

PSV increased in the CT (P = .004) and FT (P = .004) groups after intervention (Figure1C). The increase in PSV was not significantly different between CT and FT (P = .968). The study also found a decrease in SI in the CT (P = .006) and FT (P = .028) groups when compared to CO (Figure1D). However, there was no significant difference observed in the reduction of SI between CT and FT groups (P = .509). Also, the analysis of miR-126 and miR-1 gene expression between groups, showed that values in the FT and CT was significantly different compared to each other (P=0.001).

Discussion

The findings revealed a significant reduction of weight in CT and FT groups. Both concurrent and functional training can increase muscle mass and cellular metabolism, leading to an increased energy demand for maintaining muscle tissue. This contributes to an increase in resting metabolic rate, which can lead to a greater caloric burn even at rest. Moreover, exercise training can stimulate the release of myokines such as irisin, which can promote the browning of white adipose tissue (WAT) into more thermogenic beige adipose tissue (BAT) (Jiménez-Aranda et al. 2013). This process increases the energy expenditure and contributes to weight loss.

Also, brachial artery FMD increased in the CT and FT groups. Concurrent training can upregulate AMP-activated protein kinase (AMPK) activity, leading to an increased glucose uptake and fatty acid oxidation in skeletal muscles (Methenitis 2018). AMPK activation can also downregulate adipogenesis and promote lipolysis, which can contribute to a reduction in body weight (Liou et al. 2015). The cellular cascade in which concurrent and functional training increase FMD, or flow-mediated dilation, involves a number of processes. Essentially, when we engage in physical activity, it triggers a number of cellular responses that work together to improve our overall cardiovascular health. The increased demand for oxygen and blood flow to the muscles prompts the release of nitric oxide, a vasodilator that helps to widen blood vessels and improve blood flow (Premont et al. 2021). Functional training, which involves exercises that mimic real-life movements and activities, also appears to contribute to improve FMD. This is likely due to the fact that functional training

engages a wider range of muscles and movement patterns, which helps to enhance overall cardiovascular fitness.

PSV, or peak systolic velocity, also increased following FT and CT. Increasing PSV cardio is an important goal for improving cardiovascular health, as it indicates improved blood flow and circulation (Ioakeimidis et al. 2016). Exercise training can be effective for improving PSV cardio (Zanetti et al. 2020). It has been shown that concurrent training helps to increase the overall strength and contractility of the heart (Wong et al. 2010), while endurance exercise improves the overall efficiency of the cardiovascular system (Sanchis-Gomar et al. 2022). At the cellular level, concurrent training triggers a number of different processes that ultimately lead to improved PSV cardio. These processes include: Increased angiogenesis and nitric oxide production, and improved mitochondrial function (Brisebois 2019). Additionally, exercise training can also lead to adaptations in the smooth muscle cells of the arteries, which can enhance their ability to contract and relax in response to changes in blood flow (Gliemann et al. 2019). These adaptations help to maintain healthy blood pressure and improve arterial function, ultimately leading to increased PSV.

The study found a decrease in SI in both experimental groups. Arterial stiffness is related to the accumulation of advanced glycation end products (AGEs) in the extracellular matrix of blood vessels (Birukov et al. 2021). AGEs form from the reaction of glucose and other reducing sugars with proteins, lipids, and nucleic acids, which alters their structure and function (Parwani and Mandal 2023). This process is known as glycation and is accelerated in conditions of hyperglycemia, oxidative stress, and inflammation (Bavkar et al. 2019). The cellular cascade that leads to arterial stiffness involves several pathways, including the production of reactive oxygen species (ROS), the activation of pro-inflammatory cytokines, and the upregulation of matrix metalloproteinases (MMPs)s (Massaro et al. 2019). ROS can damage cellular components and induce oxidative stress, which triggers the expression of endothelial adhesion molecules and chemokines, recruiting leukocytes to the site of injury (Batty, Bennett, and Yu 2022). The MMPs can degrade the extracellular matrix, leading to structural disruptions and mechanical alterations (Winkler et al. 2020). Inflammation is a key driver of atherosclerosis and arterial stiffness (Türkuçar et al. 2021). Exercise can reduce inflammation by decreasing the expression of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), and increasing the production of anti-inflammatory cytokines (Shirvani et al. 2021). Exercise can reduce oxidative stress by upregulating the expression of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, and decreasing the production of ROS (Flensted-Jensen et al. 2021).

Present study showed an increase in gene expression of miR-1. The cellular cascade that leads to increased miR-1 expression in response to functional and concurrent training is complex and not fully understood. However, it is thought to involve multiple signaling pathways that are activated by different stimuli during exercise. Specifically, exercise-induced mechanical stress and metabolic stress have been shown to activate distinct signaling pathways that converge on a common downstream target, which is thought to be responsible for the increased miR-1 expression (Saito et al. 2020). It is also thought that other factors, such as changes in gene expression and protein synthesis, may contribute to the cellular cascade that leads to increased miR-1 expression. Also, the increased blood flow can stimulate the release of a protein called PGC-1α, which regulates gene transcription. Specifically, PGC-1α activates transcription factors that bind to the promoter region of the miR-1 gene, leading to an increase in miR-1 gene expression (Ren et al. 2020).

On the other hand, it was found that miR-126 gene expression, which plays a key role in the formation of new blood vessels, significantly elevated after interventions. A possible cellular mechanism for this finding could involve the activation of signaling pathways associated with exercise-induced adaptations. During exercise, muscle cells experience mechanical stress and metabolic changes, leading to the activation of various signaling pathways. One potential pathway involved in the upregulation of miR-126 expression is the PI3K/Akt pathway (Lou et al. 2022). In response to exercise, the PI3K/Akt pathway can be activated, leading to downstream effects on gene expression. Akt, a key protein in this pathway, has been shown to regulate the expression of microRNAs, including miR-126. Activation of Akt can promote the transcription of miR-126, leading to its increased expression (Wang et al. 2019). Also, miR-126 is known to play a role in angiogenesis, which is the formation of new blood vessels (Wang et al. 2008). It is possible that the increased miR-126 expression observed after training could be involved in the adaptation of muscle cells to exercise-induced angiogenesis.

Conclusions

In conclusion, during menopause, there is a decrease in estrogen levels, which can lead to an increased risk of cardiovascular diseases (Hashemzadeh et al. 2020). Decreased estrogen levels during menopause can lead to an impaired mRNAs expression, which can have a negative effect on the cardiovascular system. Postmenopausal women are recommended to engage in regular exercise and maintain a healthy lifestyle to reduce the risk of such diseases. Therefore, incorporating exercise interventions can yield positive effects such as promoting gene expression of mir1 and miR-126 and therefore could be considered as a safe and effective means of improving the overall health and well-being of menopausal women.

What is already known on this subject?

Physical activity is essential for maintaining a healthy cardiovascular system, which consists of the heart, blood vessels, and blood. By engaging in regular exercise, people can improve the function and structure of their cardiovascular system, as well as prevent or treat various diseases that affect it. Some of the positive effects of physical activity include reducing oxidative stress and inflammation, enhancing insulin sensitivity and metabolism, promoting stem cell repair, strengthening cellular energy production, increasing vascular dilation and blood flow, and enlarging and strengthening the heart muscle. Therefore, physical activity can help people achieve optimal cardiovascular health and well-being.

What this study adds?

Engaging in functional and concurrent exercise training can have beneficial outcomes, such as enhancing the levels of mir1 and miR-126, two types of microRNAs that are involved in regulating angiogenesis and inflammation, increasing PSV and FMD, two measures of vascular function that reflect the ability of blood vessels to dilate and respond to changes in blood flow, and lowering Stifnex index, a measure of arterial stiffness that indicates the risk of cardiovascular disease. Therefore, exercise can be regarded as a safe and effective way to improve the overall health and well-being of women who are going through menopause.

Organ Cross-Talk Tips:

- Decreased estrogen levels during menopause can lead to an impaired mRNAs expression, which can have a negative effect on the cardiovascular system.

Acknowledgements

We gratefully thank the subjects for their participation in this study.

Funding

None.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the Ethical Standards of the Institutional and/or National Research Committee (IR.SSRI.REC.1401.177). Informed consent was obtained from all participants.

Informed consent Informed consent was taken from all subjects.

Author contributions

Conceptualization: M.K, F.S, H.S, L.G.; Methodology: M.K, F.S, H.S, L.G.; Software: M.K, F.S, H.S, L.G.; Validation: M.K, F.S, H.S, L.G.; Formal analysis; M.K, F.S, H.S, L.G.; Investigation: M.K, F.S, H.S, L.G.; Resources: M.K, F.S, H.S, L.G.; Data curation: M.K, F.S, H.S, L.G.; Writing - original draft: M.K, F.S, H.S, L.G.; Writing – review & editing: M.K, F.S, H.S, L.G.; Visualization: M.K, F.S, H.S, L.G.; Supervision: L.G.; Project administration: L.G..; Funding acquisition: M.K.

References

Ai, Jing, Rong Zhang, Yue Li, Jielin Pu, Yanjie Lu, Jundong Jiao, Kang Li, Bo Yu, Zhuqin Li, and Rongrong Wang. 2010. 'Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction', Biochemical and Biophysical Research Communications, 391: 73-77. DOI: https://doi.org/10.1016/j.bbrc.2009.11.005

Aliyeva, Amina M, Natalya V Teplova, Aleksey V Butenko, Evgeniy E Averin, Madina F Akhmedova, Yulia A Shikhova, Ramiz K Valiev, Muhammetsakhet N Sariyev, Irina A Kotikova, and Igor G Nikitin. 2023. 'MicroRNA-1 and microRNA-133: Small molecules of great importance in the aspect of cardiovascular diseases', Therapy, 9: 72-79. DOI: https://doi.org/10.1080/13813455.2021.1880995

Batty, Matthew, Martin R Bennett, and Emma Yu. 2022. 'The role of oxidative stress in atherosclerosis', Cells, 11: 3843. DOI: https://doi.org/10.3390/cells11153843

Bavkar, Laxman N, Rahul S Patil, Sheetalnath B Rooge, Megha L Nalawade, and Akalpita U Arvindekar. 2019. 'Acceleration of protein glycation by oxidative stress and comparative role of antioxidant and protein glycation inhibitor', Molecular and Cellular Biochemistry, 459: 61-71. DOI: https://doi.org/10.1007/s11010-019-03581-0

Bays, Harold E, Pam R Taub, Elizabeth Epstein, Erin D Michos, Richard A Ferraro, Alison L Bailey, Heval M Kelli, Keith C Ferdinand, Melvin R Echols, and Howard Weintraub. 2021. 'Ten things to know about ten cardiovascular disease risk factors', American Journal of Preventive Cardiology, 5: 100149. DOI: https://doi.org/10.1016/j.ajpc.2020.100149

Biglia, Nicoletta, Luca Mariani, Luca Sgro, Paola Mininanni, Giulia Moggio, and Piero Sismondi. 2007. 'Increased incidence of lobular breast cancer in women treated with hormone replacement therapy: implications for diagnosis, surgical and medical treatment', Endocrine-related cancer, 14: 549-67. DOI: https://doi.org/10.1677/ERC-07-0074

Birukov, Anna, Rafael Cuadrat, Elli Polemiti, Fabian Eichelmann, and Matthias B Schulze. 2021. 'Advanced glycation end-products, measured as skin autofluorescence, associate with vascular stiffness in diabetic, pre-diabetic and normoglycemic individuals: a cross-sectional study', Cardiovascular Diabetology, 20: 1-11. DOI: https://doi.org/10.1186/s12933-021-01224-8

Brisebois, Matthew. 2019. Acute Effects of Concurrent Exercise on Biomarkers of Angiogenesis and Cardioprotection in Sedentary Adults (Texas Woman's University). No DOI available

Carresi, Cristina, Rocco Mollace, Roberta Macrì, Miriam Scicchitano, Francesca Bosco, Federica Scarano, Anna Rita Coppoletta, Lorenza Guarnieri, Stefano Ruga, and Maria Caterina Zito. 2021. 'Oxidative stress triggers defective autophagy in endothelial cells: role in atherothrombosis development', Antioxidants, 10: 387. DOI: https://doi.org/10.3390/antiox10030387

Dilsiz, Nihat. 2020. 'Role of exosomes and exosomal microRNAs in cancer', Future science OA, 6: FSO465. DOI: https://doi.org/10.2144/fsoa-2019-0128

Duarte, Filipe V, Carlos M Palmeira, and Anabela P Rolo. 2014. 'The role of microRNAs in mitochondria: small players acting wide', Genes, 5: 865-86. DOI: https://doi.org/10.3390/genes5040865

Flensted-Jensen, Mathias, Martin Gram, Flemming Dela, Jørn Wulff Helge, and Steen Larsen. 2021. 'Six weeks of high intensity cycle training reduces H2O2 emission and increases antioxidant protein levels in obese adults with risk factors for type 2 diabetes', Free Radical Biology and Medicine, 173: 1-6. OI: https://doi.org/10.1016/j.freeradbiomed.2021.06.001

Gliemann, Lasse, Camilla Vestergaard Hansen, Nicolai Rytter, and Ylva Hellsten. 2019. 'Regulation of skeletal muscle blood flow during exercise', Current Opinion in Physiology, 10: 146-55. DOI: https://doi.org/10.1016/j.cophys.2019.07.003

Gupta, Shashi K, Claudia Bang, and Thomas Thum. 2010. 'Circulating microRNAs as biomarkers and potential paracrine mediators of cardiovascular disease', Circulation: Cardiovascular Genetics, 3: 484-88. DOI: https://doi.org/10.1161/CIRCGENETICS.110.957415

Hartono, Felicia A, Patrick W Martin-Arrowsmith, Wouter M Peeters, and Tyler A Churchward-Venne. 2022. 'The effects of dietary protein supplementation on acute changes in muscle protein synthesis and longer-term changes in muscle mass, strength, and aerobic capacity in response to concurrent resistance and endurance exercise in healthy adults: a systematic review', Sports Medicine, 52: 1295-328. DOI: https://doi.org/10.1007/s40279-022-01560-4

Hashemzadeh, Mehrnoosh, Ryan Romo, Joseph M Arreguin, and Mohammed Reza Movahed. 2020. 'The effects of estrogen and hormone replacement therapy on cardiovascular systems', Future cardiology, 17: 347-53. DOI: https://doi.org/10.2217/fca-2020-0013

Hibbs, Angela E, Kevin G Thompson, Duncan French, Allan Wrigley, and Iain Spears. 2008. 'Optimizing performance by improving core stability and core strength', Sports Medicine, 38: 995-1008. DOI: https://doi.org/10.2165/00007256-200838120-00004

Ioakeimidis, Nikolaos, Charalambos Vlachopoulos, Konstantinos Rokkas, Zisis Kratiras, Athanasios Angelis, Alexis Samentzas, Charalambos Fassoulakis, and Dimitris Tousoulis. 2016. 'Dynamic penile peak systolic velocity predicts major adverse cardiovascular events in hypertensive patients with erectile dysfunction', Journal of Hypertension, 34: 860-68. DOI: https://doi.org/10.1097/HJH.0000000000000858

Jian, Bin, Zhen Li, Tao Zhang, Li Guo Tan, and Can Can Qiang. 2021. 'The Expression Changes and Clinical Significance of Serum miRNA-1 in Patients with Heart Failure', Indian Journal of Pharmaceutical scien-ces: 111-15. DOI: https://doi.org/10.36468/pharmaceutical-sciences.803

Jiménez-Aranda, Aroa, Gumersindo Fernández-Vázquez, Daniel Campos, Mohamed Tassi, Lourdes Velasco-Perez, Dun-Xian Tan, Russel J Reiter, and Ahmad Agil. 2013. 'Melatonin induces browning of inguinal white adipose tissue in Zucker diabetic fatty rats', Journal of pineal research, 55: 416-23. DOI: https://doi.org/10.1111/jpi.12089

Kalyani, Rita Rastogi, Mark Corriere, and Luigi Ferrucci. 2014. 'Age-related and disease-related muscle loss: the effect of diabetes, obesity, and other diseases', The lancet Diabetes & endocrinology, 2: 819-29. DOI: https://doi.org/10.1016/S2213-8587(14)70034-8

Ko, Seong-Hee, and Hyun-Sook Kim. 2020. 'Menopause-associated lipid metabolic disorders and foods beneficial for postmenopausal women', Nutrients, 12: 202. DOI: https://doi.org/10.3390/nu12010202

Kura, Branislav, Mihir Parikh, Jan Slezak, and Grant N Pierce. 2019. 'The influence of diet on microRNAs that impact cardiovascular disease', Molecules, 24: 1509. DOI: https://doi.org/10.3390/molecules24081509

Leite, Richard D, J Prestes, GB Pereira, GE Shiguemoto, and SEA Perez. 2010. 'Menopause: highlighting the effects of resistance training', International journal of sports medicine, 31: 761-67. DOI: https://doi.org/10.1055/s-0030-1261893

Liou, Chian-Jiun, Xuan-Yu Lai, Ya-Ling Chen, Chia-Ling Wang, Ciao-Han Wei, and Wen-Chung Huang. 2015. 'Ginkgolide C suppresses adipogenesis in 3T3-L1 adipocytes via the AMPK signaling pathway', Evidence-Based Complementary and Alternative Medicine, 2015. DOI: https://doi.org/10.1155/2015/915467

Liu, Chiung-Ju, and Nancy Latham. 2011. 'Can progressive resistance strength training reduce physical disability in older adults? A meta-analysis study', Disability and rehabilitation, 33: 87-97. DOI: https://doi.org/10.3109/09638288.2010.503835

Liu, Chiung-ju, Deepika M Shiroy, Leah Y Jones, and Daniel O Clark. 2014. 'Systematic review of functional training on muscle strength, physical functioning, and activities of daily living in older adults', European review of aging and physical activity, 11: 95-106. DOI: https://doi.org/10.1007/s11556-014-0144-1

Lou, Zhe, Qiaobei Li, Chunyan Wang, and Yinyan Li. 2022. 'The effects of microRNA-126 reduced inflammation and apoptosis of diabetic nephropathy through PI3K/AKT signalling pathway by VEGF', Archives of physiology and biochemistry, 128: 1265-74. DOI: https://doi.org/10.1080/13813455.2021.1880994

Lunenfeld, Bruno, and Pamela Stratton. 2013. 'The clinical consequences of an ageing world and preventive strategies', Best practice & research Clinical obstetrics & gynaecology, 27: 643-59. DOI: https://doi.org/10.1016/j.bpobgyn.2013.05.005

Madika, Anne-Laure, Phenicia Nasserdine, Sandra Langlet, Charlotte Lecerf, Guillaume Ledieu, Patrick Devos, and Claire Mounier-Vehier. 2019. 'Association between reproductive factors and carotid atherosclerosis in post-menopausal women', Maturitas, 126: 38-44. DOI: https://doi.org/10.1016/j.maturitas.2019.05.004

Massaro, Marika, Egeria Scoditti, Maria Annunziata Carluccio, and Raffaele De Caterina. 2019. 'Oxidative stress and vascular stiffness in hypertension: A renewed interest for antioxidant therapies?', Vascular pharmacology, 116: 45-50. DOI: https://doi.org/10.1016/j.vph.2019.03.002

McKinlay, Sonja M, Donald J Brambilla, and Jennifer G Posner. 1992. 'The normal menopause transition', Maturitas, 14: 103-15. DOI: https://doi.org/10.1016/0378-5122(92)90003-D

Methenitis, Spyridon. 2018. 'A brief review on concurrent training: from laboratory to the field', Sports, 6: 127. DOI: 10.3390/sports6040127

Montenont, Emilie, Matthew T Rondina, and Robert A Campbell. 2019. 'Altered functions of platelets during aging', Current opinion in hematology, 26: 336. DOI: https://doi.org/10.1097/MOH.0000000000000530

Parwani, Kirti, and Palash Mandal. 2023. 'Role of advanced glycation end products and insulin resistance in diabetic nephropathy', Archives of physiology and biochemistry, 129: 95-107. DOI: https://doi.org/10.1080/13813455.2021.1880995

Premont, Richard T, James D Reynolds, Rongli Zhang, and Jonathan S Stamler. 2021. 'Red blood cell-mediated S-nitrosohemoglobin-dependent vasodilation: lessons learned from a β-globin Cys93 knock-in mouse', Antioxidants & Redox Signaling, 34: 936-61. DOI: https://doi.org/10.1089/ars.2020.8132

Ren, Shuyue, Peirong Lin, Jingrong Wang, Haoying Yu, Tingting Lv, Lan Sun, and Guanhua Du. 2020. 'Circular RNAs: promising molecular biomarkers of human aging-related diseases via functioning as an miRNA sponge', Molecular Therapy-Methods & Clinical Development, 18: 215-29. DOI: https://doi.org/10.1016/j.omtm.2020.07.016

Rossi, Fabrício Eduardo, Ana Claudia S Fortaleza, Lucas M Neves, Tiego A Diniz, Marcela R De Castro, Camila Buonani, Jorge Mota, and Ismael F Freitas. 2017. 'Combined training (strength plus aerobic) potentiates a reduction in body fat but only functional training reduced low-density lipoprotein cholesterol in postmenopausal women with a similar training load', Journal of exercise rehabilitation, 13: 322. DOI: https://doi.org/10.12965/jer.1734998.499

Saito, Yuki, Takako S Chikenji, Takashi Matsumura, Masako Nakano, and Mineko Fujimiya. 2020. 'Exercise enhances skeletal muscle regeneration by promoting senescence in fibro-adipogenic progenitors', Nature communications, 11: 889. DOI: https://doi.org/10.1038/s41467-020-14676-4

Sanchis-Gomar, Fabian, Carl J Lavie, Jorge Marín, Carme Perez-Quilis, Thijs MH Eijsvogels, James H O'Keefe, Marco V Perez, and Steven N Blair. 2022. 'Exercise effects on cardiovascular disease: from basic aspects to clinical evidence', Cardiovascular Research, 118: 2253-66. DOI: https://doi.org/10.1093/cvr/cvab237

Shirvani, Hossein, Reza Mirnejad, Mehdi Soleimani, and Ehsan Arabzadeh. 2021. 'Swimming exercise improves gene expression of PPAR-γ and downregulates the overexpression of TLR4, MyD88, IL-6, and TNF-α after high-fat diet in rat skeletal muscle cells', Gene, 775: 145441. DOI: https://doi.org/10.1016/j.gene.2020.145441

Soci, Ursula Paula Renó, Stephano Freitas Soares Melo, João Lucas Penteado Gomes, André Casanova Silveira, Clara Nóbrega, and Edilamar Menezes de Oliveira. 2017. 'Exercise training and epigenetic regulation: multilevel modification and regulation of gene expression', Exercise for Cardiovascular Disease Prevention and Treatment: From Molecular to Clinical, Part 2: 281-322. DOI: https://doi.org/10.1007/978-981-10-4307-9_1

Stenger, Leslie. 2018. 'What is functional/neuromotor fitness?', ACSM's Health & Fitness Journal, 22: 35-43. DOI: https://doi.org/10.1249/FIT.0000000000000430

Tan, DA, and ARB Dayu. 2022. 'Menopausal hormone therapy: why we should no longer be afraid of the breast cancer risk', Climacteric, 25: 362-68. DOI: https://doi.org/10.1080/13697137.2021.1975736

Thijssen, Dick HJ, Mark A Black, Kyra E Pyke, Jaume Padilla, Greg Atkinson, Ryan A Harris, Beth Parker, Michael E Widlansky, Michael E Tschakovsky, and Daniel J Green. 2011. 'Assessment of flow-mediated dilation in humans: a methodological and physiological guideline', American Journal of Physiology-Heart and Circulatory Physiology, 300: H2-H12. DOI: https://doi.org/10.1152/ajpheart.00471.2010

Türkuçar, Serkan, Kaan Yıldız, Tuncay Küme, Ceyhun Açarı, Hatice Adıgüzel Dundar, Balahan Makay, Mustafa Kır, and Erbil Ünsal. 2021. 'Does Familial Mediterranean Fever Provoke Atherosclerosis in Children? Evaluation of Arterial Stiffness and Serum Endocan Levels', Clinical Rheumatology, 40: 4199-206. DOI: https://doi.org/10.1007/s10067-021-05776-8

Wang, An, Bo Sun, Jia Qiu, Shigang Qiao, and Chen Wang. 2016. 'Role of microRNA-1 on fibrosis induced by high glucose cultured cardiac fibroblasts', Chinese Critical Care Medicine: 1113-17. OI: https://doi.org/10.3760/cma.j.issn.2095-4352.2016.12.006

Wang, H-F, Y-Q Wang, L Dou, H-M Gao, B Wang, N Luo, and Y Li. 2019. 'Influences of up-regulation of miR-126 on septic inflammation and prognosis through AKT/Rac1 signaling pathway', European Review for Medical & Pharmacological Sciences, 23. DOI: https://doi.org/10.26355/eurrev_201912_19764

Wang, Shusheng, Arin B Aurora, Brett A Johnson, Xiaoxia Qi, John McAnally, Joseph A Hill, James A Richardson, Rhonda Bassel-Duby, and Eric N Olson. 2008. 'The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis', Developmental cell, 15: 261-71. DOI: https://doi.org/10.1016/j.devcel.2008.07.002

Wilson, Jacob M, Pedro J Marin, Matthew R Rhea, Stephanie MC Wilson, Jeremy P Loenneke, and Jody C Anderson. 2012. 'Concurrent training: a meta-analysis examining interference of aerobic and resistance exercises', The Journal of Strength & Conditioning Research, 26: 2293-307. DOI: https://doi.org/10.1519/JSC.0b013e31823a3e2d

Winkler, Juliane, Abisola Abisoye-Ogunniyan, Kevin J Metcalf, and Zena Werb. 2020. 'Concepts of extracellular matrix remodelling in tumour progression and metastasis', Nature communications, 11: 5120. DOI: https://doi.org/10.1038/s41467-020-18961-w

Wong, Pui-lam, Anis Chaouachi, Karim Chamari, Alexandre Dellal, and Ulrik Wisloff. 2010. 'Effect of preseason concurrent muscular strength

and high-intensity interval training in professional soccer players', The Journal of Strength & Conditioning Research, 24: 653-60. DOI: https://doi.org/10.1519/JSC.0b013e3181c06a77

Xiang, Du, Yang Liu, Shujun Zhou, Encheng Zhou, and Yanfeng Wang. 2021. 'Protective effects of estrogen on cardiovascular disease mediated by oxidative stress', Oxidative Medicine and Cellular Longevity, 2021: 1-15. DOI: https://doi.org/10.1155/2021/8817950

Zanetti, Hugo Ribeiro, Alexandre Goncalves, Leandro Teixeira Paranhos Lopes, Edmar Lacerda Mendes, Leonardo Roever, Mario Leon Silva-Vergara, Fernando Freitas Neves, and Elmiro Santos Resende. 2020. 'Effects of exercise training and statin use in people living with human immunodeficiency virus with dyslipidemia', Medicine & Science in Sports & Exercise, 52: 16-24. DOI: https://doi.org/10.1249/MSS.0000000000002248