Journal of Molecular and Cellular Cardiology Plus 2 (2022) 100026

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology Plus

journal homepage: https://www.journals.elsevier.com/journal-of-molecular-and-cellular-cardiology-plus

The effect of moderate intensity aerobic exercise on cardiovascular function, cardiorespiratory fitness and estrogen receptor alpha gene in overweight/obese postmenopausal women: A randomized controlled trial

Abbas Malandish [a,*], Mohammad Rahmati-Yamchi [b]

[a] Department of Exercise Physiology, Faculty of Sport Sciences, Urmia University, Urmia, Iran
[b] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

ARTICLE INFO

Keywords:
Cardiovascular system
Aerobic training
Estrogen receptor alpha gene
Menopause
Obesity

ABSTRACT

Objective: The purpose of this study was to examine the effect of 12 weeks of moderate intensity aerobic exercise on echocardiographic and cardiorespiratory fitness (CRF) parameters, lymphocyte estrogen receptor alpha (ERα) gene expression and sex hormones (17β-estradiol and progesterone) in overweight/obese postmenopausal women (OPMW).
Methods: Twenty-seven sedentary OPMW aged 45 to 65 years old were randomly assigned to exercise (EX, n = 14) and control (C, n = 13) groups. The EX group performed warm up-walking/jogging moderate intensity aerobic exercise program- recovery (60 min/day, 3 days/week at 70 % of maximal heart rate reserve for 12 weeks) while the C group participated in no intervention and maintained their daily physical activity level, sedentary normal lifestyle and dietary habits during 12-week. The lymphocyte ERα gene expression, serum levels of 17β-estradiol and progesterone, and CRF & echocardiographic parameters were measured at baseline and week-12.
Results: After 12-week, the increase in ERα gene expression (p = 0.009, estimate of effect size/Eta = 28.2 %), VO_{2max} (p = 0.001, Eta = 53.4 %), walking-jogging time to exhaustion (WJTE) (p = 0.001, Eta = 55.1 %), metabolic equivalent of task (METs) (p = 0.001, Eta = 97.9 %), left ventricular ejection fraction (LVEF) (p = 0.001, Eta = 53.6 %), cardiac output (Q) (p = 0.036, Eta = 22.3 %), and cardiac index (p = 0.030, Eta = 22.5 %) were significantly higher in the EX group compared to the C group, whereas body fat (p = 0.023, Eta = 25.7 %), left ventricular end-systolic diameter (LVESD) (p = 0.013, Eta = 28.3 %), and mitral E-wave deceleration time (E-wave D time) (p = 0.007, Eta = 32.1 %) were significantly decreased.
Conclusions: The results suggested that moderate intensity aerobic exercise can be improved cardiac function such as LVEF, Q, cardiac index, LVESD, and E-wave D time, CRF, ERα-mRNA gene expression as well as maintained sex hormones among sedentary OPMW during menopause, as these positive cellular and molecular or physiological adaptations may be signs of cardioprotective effects by aerobic exercise.

1. Introduction

Cardiovascular disease (CVD) is the most common cause of mortality in older people [1,2]. Aging-induced events such as CVDs among overweight/obese postmenopausal women (OPMW) have been identified as a global health problem [1]. In addition, sedentary-induced disorders are other factors that affect the cardiorespiratory system (CRS) and cardiovascular system (CVS) during menopause [3]. Beside the genetic and environmental factors affecting CVS, aerobic exercise and cellular & molecular mechanisms are also involved in CVS, including estrogen receptor α (ERα) as a messenger of cardiomyocytes and sex hormones [4]. On the other hand, there are both ERα and ERβ in different cardiac cells, including cardiomyocytes, cardiac fibroblast cells, endothelial cells, and smooth muscle cells in humans [4–6]. It seems that decreased sex hormones, including estrogen/ERα and progesterone in menopause lead to disturbances in the cardiorespiratory regulatory systems such as CVS and sympathetic nervous system (SNS) [1,3,7]. The aerobic exercise is an important non-medication factor in old women to take care of their health and CVS later in life span [1,8]. In other words, aerobic exercise intervention is one of the most effective ways of reducing risk factors of CVDs and mortality [1,9,10]. Recent studies

Abbreviations: CVD, Cardiovascular disease; CRS, Cardiorespiratory system; CVS, Cardiovascular system; ERα, Estrogen receptor α; ERβ, Estrogen receptor β; SNS, Sympathetic nervous system; mRNA, Messenger ribonucleic acid; CRF, Cardiorespiratory fitness; RCT, Randomized controlled trial; ECG, Electrocardiogram; METs, Metabolic equivalent of tasks; VO_{2max}, Maximal oxygen uptake; GXT, Graded exercise treadmill test; RPE, Rating of perceived exertion; HRmax, Maximal heart rate; DXA, Dual-energy X-ray absorptiometry; ELISA, Enzyme-linked immunosorbent assay; EDTA, Ethylenediaminetetra-acetic acid; cDNA, Complementary deoxyribonucleic-acid; RGDE, Rapid genomic DNA extraction; qRT-PCR, Quantitative real-time reverse transcription polymerase chain reaction; WJTE, Walking-jogging time to exhaustion; E-wave D time, E-wave deceleration time; LVEF, Left ventricular ejection fraction; Q, Cardiac output.

* Corresponding author at: No. 19, Shams Tabrizi St., Velayat Ave., Keikhali Zone, Yamchi, East Azerbaijan, Iran.
E-mail address: malandish@gmail.com (A. Malandish).

http://dx.doi.org/10.1016/j.jmccpl.2022.100026
Received 8 October 2022; Received in revised form 26 November 2022; Accepted 27 November 2022

have shown that exercise enhances the physiological myocardial hypertrophy adaptations through the cellular and molecular mechanism of ERs in animal models [4,11]. Recently, a study reported that an increase in the total ERα expression represents a compensatory process in the stability of cardiac intercalated discs in humans [5]. In addition, a recent study reported that up-regulation of ERα-messenger ribonucleic acid (mRNA) detected in the myocardium of human patients with congenital cardiac disease was associated with lower myocardial damage and lower cytolysis in the early post-operative period might support the assumed cardioprotective role of ERα in humans [12]. However, the role of ERα gene expression and cell signaling cascades are not fully understood in CVS following exercise intervention and there are currently no studies regarding the effect of aerobic exercise on gene expression of human ERα in scientific databases. Therefore, considering the positive physiological impact of aerobic training on cardiorespiratory function, and the possibility of stimulating the lymphocyte ERα gene expression, sex hormones, and as well as its effects on improved cardiac function and echocardiographic parameters, we hypothesized that 1) aerobic exercise intervention would be useful in the improvement of cardiac function, echocardiographic parameters and as well as cardiorespiratory fitness (CRF) in OPMW; and 2) aerobic exercise intervention would be useful in stimulating the lymphocyte ERα-mRNA expression and serum levels of sex hormones (17β-estradiol and progesterone) in OPMW. Thus, we conducted a randomized controlled trial to determine the impact of 12 weeks of moderate intensity aerobic exercise intervention or sedentary lifestyle on cardiac function, CRF, ERα gene expression as well as serum levels of sex hormones in OPMW. To our knowledge, this is the first study to address this subject in OPMW.

2. Materials and methods

2.1. Participants

This randomized controlled trial (RCT) study was approved by the Ethics Committee of biomedical sciences at Urmia University of medical sciences according to the Helsinki declaration and was conducted in January 2016 at Urmia University, Iran. Twenty-seven sedentary OPMW volunteered for this study (Fig. 1). The inclusion criteria of participants included (1) OPMW with the age range of 45 to 65 years were spent at least 1 year and <5 years at menopause, (2) having body mass index (BMI) above 25 kg/m^2, (3) free of medication (4) no history of CVDs, as well as any clinical problems such as hypertension, electrocardiogram (ECG) or echocardiography abnormalities, diabetes mellitus, fatty liver disease, pulmonary disease, gastrointestinal disease, and neurologic diseases, and (5) no history of regular exercise, weight gain, weight loss, hormone therapy, pharmacological intervention, smoking for at least 6 months before the start of the study as described before [13]. The CVS was evaluated based on the normal ECG (Biocare, ECG-1200, China) and Doppler echocardiography (Esaote Spa, Firenze, Italy). Exclusion criteria of participants included (1) discontinue of regular exercise protocol or normal sedentary lifestyle and/or dietary habits during 12-week, and (2) non-participation in blood sampling at baseline and/or week-12. The energy expenditure and physical activity level of OPMW were expressed in terms of metabolic equivalent of tasks (METs), as described before [13]. All participants attended the exercise venue and received information about the protocols and procedures, as well as the possible risks and benefits involved in the study. Informed written consent was obtained from all the participants. The participants were then randomly assigned to either exercise (EX, n = 14) or control (C, n = 13) group (Table 1). True random number generation was used for randomization of this study. The EX group performed 12 weeks of moderate intensity aerobic exercise. The C group participated in no intervention during 12 weeks and maintained their daily physical activity level, sedentary normal lifestyle and dietary habits during 12 weeks.

2.2. Aerobic exercise intervention

To determine the maximal oxygen uptake (VO_{2max}), each participant underwent a graded exercise treadmill test (GXT-Turbo Fitness, LX740, Taiwan) prior to the beginning of this study. By using the Borg scale, rating of perceived exertion (RPE) was recorded in the last 10 s of each stage. For the first 3-min stage of the GXT, participant walked up a 5 % grade at a self-

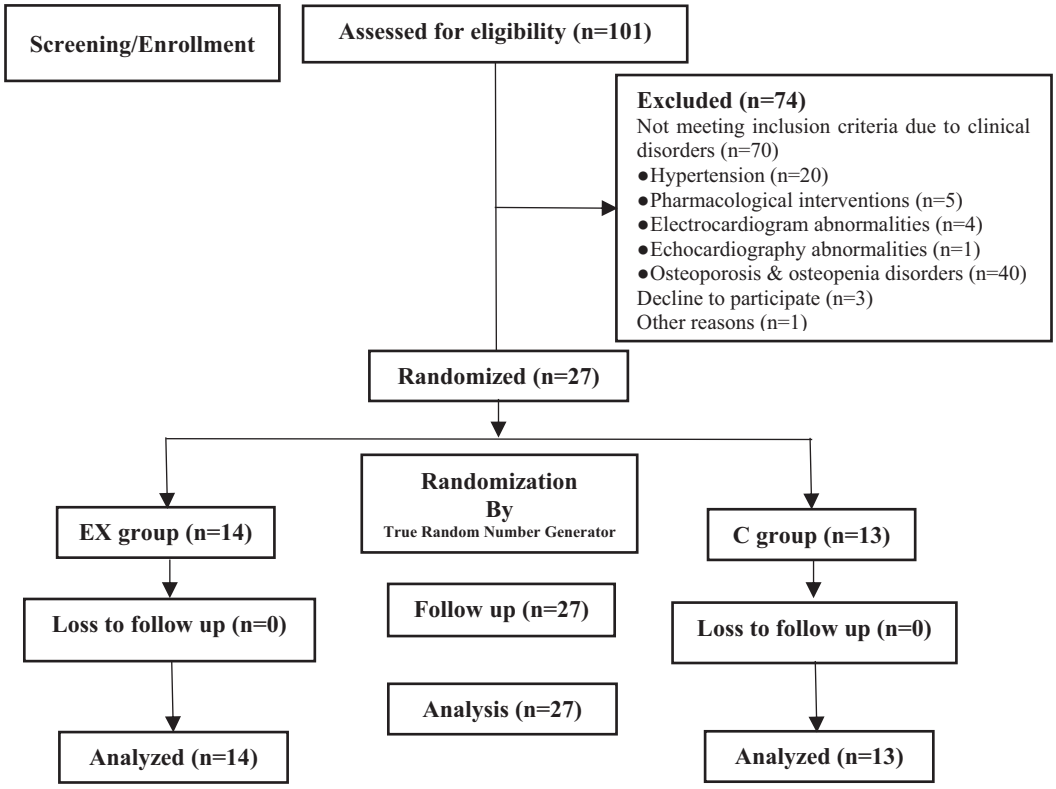

Fig. 1. Follow-up diagram in our study.

Table 1
Physiological characteristics and menopause status of OPMWs at baseline and week 12 in the EX and C groups.

Variables	EX (n = 14)	C (n = 13)	p^b value	p^c value	Eta coefficient (%)
Physiological characteristics					
Age (years)					
Baseline	53.36 ± 3.98	53.00 ± 3.26	ns	ns	ns
Week 12	53.61 ± 3.98	53.25 ± 3.26	ns		
p^a value	ns	ns			
Height (cm)					
Baseline	158.64 ± 5.18	158.40 ± 5.42	0.768	0.698	0.009 (0.9 %)
Week 12	157.55 ± 6.39	158.30 ± 5.16	0.598		
p^a value	0.666	0.591			
Weight (kg)					
Baseline	71.90 ± 9.67	76.10 ± 16.35	0.579	0.881	0.001 (0.1 %)
Week 12	72.72 ± 10.11	75.30 ± 14.73	0.740		
p^a value	0.785	0.269			
Body mass index (kg/m^2)					
Baseline	28.61 ± 4.20	30.35 ± 6.60	0.727	0.193	0.092 (9.2 %)
Week 12	30.26 ± 4.92	29.81 ± 5.58	0.845		
p^a value	0.214	0.306			
Body surface area (m^2)					
Baseline	1.78 ± 0.11	1.82 ± 0.19	0.572	0.927	0.0 (0.0 %)
Week 12	1.77 ± 0.11	1.81 ± 0.18	0.586		
p^a value	0.546	0.225			
Total body fat (%)					
Baseline	42.03 ± 5.10	40.54 ± 5.50	0.914	0.023‡	0.257 (25.7 %)
Week 12	40.86 ± 4.67	41.45 ± 5.55	0.898		
p^a value	0.029*	0.188			
Systolic blood pressure (mm Hg)					
Baseline	120.45 ± 16.35	116.80 ± 17.65	0.485	0.712	0.119 (11.9 %)
Week 12	111.63 ± 20.10	123.20 ± 14.30	0.840		
p^a value	0.375	0.087			
Diastolic blood pressure (mm Hg)					
Baseline	75.18 ± 8.84	79.00 ± 13.81	0.457	0.489	0.001 (0.1 %)
Week 12	76.81 ± 5.90	80.70 ± 10.48	0.302		
p^a value	0.601	0.354			
Resting heart rate (beats/min)					
Baseline	80.81 ± 10.94	76.72 ± 10.70	0.185	0.471	0.031 (3.1 %)
Week 12	78.54 ± 6.81	76.27 ± 9.23	0.286		
p^a value	0.305	0.658			
Metabolic equivalent of task (METs) h/week					
Baseline	11.61 ± 0.81	12.00 ± 1.13	0.350	0.001‡	0.979 (97.9 %)
Week 12	14.94 ± 1.00	11.90 ± 1.13	0.001†		
p^a value	0.001*	0.188			
Menopause status					
Menopause age (years)					
Baseline	2.27 ± 1.42	2.63 ± 1.20	0.525	ns	ns
Week 12	2.52 ± 1.42	2.88 ± 1.20	0.525		
p^a value	ns	ns			
17β-Estradiol (pg/ml)					
Baseline	31.52 ± 16.28	41.07 ± 12.60	0.202	0.122	0.117 (11.7 %)
Week 12	29.74 ± 15.36	23.84 ± 12.32	0.402		
p^a value	0.693	0.030*			
Progesterone (pg/ml)					
Baseline	0.18 ± 0.11	0.30 ± 0.15	0.085	0.494	0.026 (2.6 %)
Week 12	0.14 ± 0.08	0.20 ± 0.18	0.402		
p^a value	0.104	0.032*			

Note. EX = exercise group; C = control group; Eta = partial Eta squared or estimates of effect size; values are mean ± SD.
* $p^a < 0.05$, significantly different from baseline values by paired samples t-test (within groups, baseline vs. week 12).
† $p^b < 0.05$, significantly different baseline and also week 12 values by independent samples *t*-test (between groups, baseline EX vs. baseline C; and week12 EX vs. week 12 C).
‡ $p^c < 0.05$, significantly different between groups during the time (interaction) analyzed by univariate analysis of variance.

selected, then for the second 3-min stage, participant had the option to either continue walking (5 % grade, constant speed) or self-select a comfortable jogging pace (level grade) at a speed of between 3.4 and 5.7 miles. Participant used heart rate monitoring to indicate when they had achieved acceptable walking-jogging speeds. Following the first two stages (which served as an initial 6-min warm-up), treadmill grade was increased 1.5 % every minute (constant speed) until participant was unable to continue despite verbal encouragement. Treadmill speed and grade were considered maximal when a given speed and grade were sustained for 1 min just prior to exhaustion. Maximal heart rate (HRmax) of exercise was recorded at the end of the GXT protocol and VO_{2max} was calculated in the estimated equation as follows: VO_{2max} = 54.07 + (7.062 × female = 0 or male = 1) − (0.1938 × weight kg) + (4.47 × speed mile/h) − (0.1453 × HR bit/min). The EX group participated in 12 weeks of aerobic exercise on treadmill, 50–60 min/day, 3 days/week (150–180 min in week) at 65 %–70 % of each individual's HRmax reserve (HR_{max}Reserve). In the first week of this study, the EX group performed walking or jogging aerobic exercise at 50 % of each participant's HR_{max}Reserve. During the second and third week, the exercise intensity was increased to 60 % of HR_{max}Reserve and then was progressed to 65 % HR_{max}Reserve in the fourth to seventh week. In the last 5 weeks, human volunteers as participants were trained at 70 % HR_{max}Reserve. A schematic of aerobic exercise protocol for

this study is shown in Fig. 2. Each training session was included 10 min of prior warm-up and 5 min of cooling down (active-recovery) at the ambient temperature of 22–26 °C and relative humidity of 45 %. All exercise sessions were performed between 09:00 and 11:00 AM and participants were supervised by exercise physiologists. The C group participated in no intervention and continued their normal lifestyle, current physical activity level and dietary habits during 12-week.

2.3. Measurements

2.3.1. Physiological & echocardiographic parameters

General characteristics of age, height, weight, and body surface area (BSA) in participants were measured by ID card, wall-meter (Beurer, Germany), digital scale (Beurer, Germany), and Mosteller formula [14], respectively. BMI and total body fat (TBF) were measured by Dual-energy X-ray absorptiometry (DXA) (Hologic, USA). Diastolic & systolic blood pressure and resting heart rate were measured by indicator machine (WDF-BP001, Brisk, Germany). Echocardiographic parameters were evaluated by Doppler echocardiography (P8000, Esaote, Italy). The all physiological and echocardiographic parameters were evaluated two times at baseline and week-12 in this study.

2.3.2. Dietary assessment

Dietary data was assessed via a 3-day food record in the first and last weeks of the exercise program (over 2 weekdays and 1 weekend day) [15]. The participants were requested to maintain their normal diet during the period of study and were instructed to consume a diet as similar as possible in each sampling day. Information on the use of medications/supplements was also obtained through standard and self-reported questionnaires [15]. Nutrition and dietary data were analyzed by nutrition analysis software (Nutrition data pro™ v1.1, StarApps Co, USA) (Table 2).

Table 2
Dietary intake differences of OPMWs at baseline and week 12 in the EX and C groups.

Variables	EX (n = 14)	C (n = 13)	p^b value	p^c value
Total energy intake (kcal/day)				
Baseline	2240.88 ± 546.82	2365.12 ± 515.60	0.602	0.341
Week 12	2302.36 ± 335.22	2298.25 ± 312.63	0.966	
p^a value	0.557	0.419		
Fat (g)				
Baseline	103.11 ± 29.13	105.22 ± 32.18	0.935	0.646
Week 12	103.14 ± 26.04	101.29 ± 21.14	0.787	
p^a value	0.997	0.566		
Carbohydrate (CHO) (g)				
Baseline	252.20 ± 114.00	294.97 ± 78.48	0.345	0.389
Week 12	264.74 ± 85.26	283.18 ± 63.76	0.605	
p^a value	0.436	0.200		
Protein (g)				
Baseline	87.75 ± 23.28	78.96 ± 26.58	0.334	0.470
Week 12	89.06 ± 20.39	80.07 ± 21.33	0.243	
p^a value	0.693	0.656		

Note. EX = exercise group; C = control group; TEI = total energy intake; values are mean ± SD.
$p^a < 0.05$, significantly different from baseline values by paired samples t-test (within groups, baseline vs. week 12).
$p^b < 0.05$, significantly different baseline and also week 12 values by independent samples *t*-test (between groups, baseline EX vs. baseline C; and week12 EX vs. week 12 C).
$p^c < 0.05$, significantly different between groups during the time (interaction) analyzed by univariate analysis of variance (between groups, baseline and week 12 EX vs. baseline and week 12 C).

2.3.3. Blood sampling and assays

Following a 12-hour overnight fasting and without exercise at least 24-h, blood samples (10 ml) were taken in the early morning (between 07:30 and 08:30 AM). Blood samples (5 ml) were collected 24 h before and after the 12-week of exercise program in order to measure serum levels of sex hormones, including 17β-estradiol and progesterone in both EX and C groups. Serum levels of 17β-estradiol and progesterone were measured using an enzyme-linked immunosorbent assay (ELISA) kit (1561-DRG, Euroimmun, Germany) and (2633-DRG, Euroimmun, Germany) by ELISA device (Stat Fax®4200- Awareness Technology, USA), respectively. Another 5 ml remaining blood samples (whole blood coated to an anticoagulant ethylenediaminetetra-acetic acid [EDTA]) were used to extract RNA in the expression of ERα and β-actin genes.

2.4. RNA extraction from peripheral blood lymphocytes and complementary deoxyribonucleic-acid (cDNA) synthesis

The rapid genomic DNA extraction (RGDE) method [16] was performed on blood samples in the laboratory. Whole blood-1000 μl (1 ml) coated to an EDTA as well as 1 ml of cell lysis buffer solution were poured into RNase DNase-Free 2 ml microtube. After mixing, it was centrifuged at 6000 rpm for 2 min. The top phase was then removed, leaving a white pellet. And then RNA was extracted using the RNX-Plus RNA Extraction Kit (SinaClon, Iran) according to the manufacturer's instructions as follows: (1) added 1 ml ice cold RNX–Plus solution to 2-ml microtube containing white pellet sample, (2) vortexed 5–10 s and incubated at room temperature for 5 min, (3) added 200 μl of Chloroform, (4) mixed well for 15 s by shaking (and not vortex), (5) incubated on ice or 4 °C for 5 min, (6) centrifuged at 12,000 rpm at 4 °C for 15 min, (7) transfer the aqueous phase to new RNase-free 1.5 ml microtube, (do not disturb the mid phase) and added equal volume of Isopropanol, (8) gently mixed and incubated on ice for 15 min, (9) centrifuged the mixture at 12,000 rpm at 4 °C for 15 min, (10) discard the supernatant and added 1 ml of 75 % Ethanol, shortly vortexed to dislodge the pellet and then centrifuged at 4 °C for 8 min at 7500 rpm, (11) discarded the supernatant and let the pellet to dry at room temperature for few minutes, (12) dissolved pellet in 50 μl of DEPC treated water and then placed the tube in 55–60 °C water bath for 10–15 min. The concentration and quality or Purity of total RNA was assessed on the basis of OD260/280 ratio (ng/μl) measurements using a NanoDrop 1000 Spectrophotometer (ThermoScientific, Wilmington, USA) and electrophoresis on a 1.2 % agarose gel in 0.5 (ng/μl) ethidium

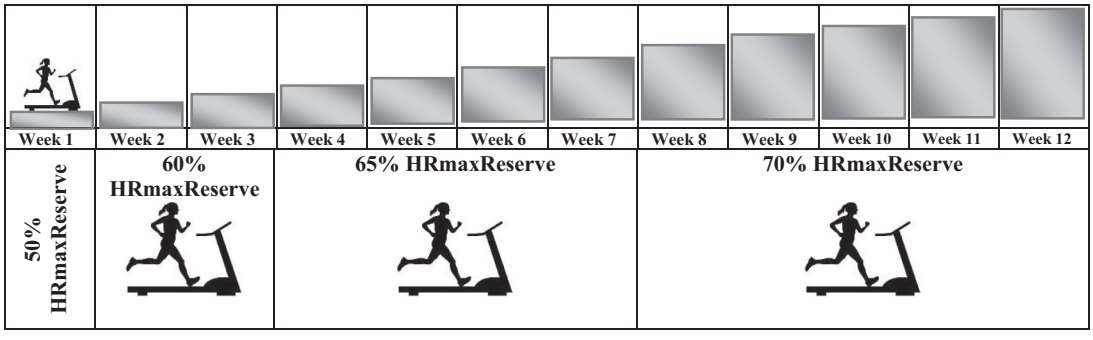

Fig. 2. A schematic of the moderate-intensity aerobic exercise protocol in our study. 50–60 min/day, 3 days/week (150–180 min in week).

bromides, respectively. The samples of RNA were converted to cDNA after treating with DNase I to eliminate any genomic DNA contamination. RNA reverse transcription (RT) was performed in a final volume of 20 (μl) reaction mixtures containing 1 (μl) template RNA, 1 (μl) dN6 primer, and 18 (μl) DEPC-DNase water by AccuPower® RocketScript™ RT-PreMix cDNA kit (k-2101, Bioneer, Germany). The reactions on the samples were performed by real time PCR (E750, ThermoScientific, Belgium) according to the manufacturer's protocol. The synthesized cDNA samples were immediately stored at −70 °C for using expression of ERα and β-actin genes.

2.5. Primer design

The specific primers of genes ERα and β-actin were used from Malandish et al. study [17] and were checked by primer Blast and OligoAnalyzer idt tools. Then, for the preparation of primers, their lyophilized powders were prepared by Bioneer Company, Daejeon from South Korea. The sequences of primers used for the ERα cDNA amplification were F-GCCAGCAGGTGCCCTACTAC and R-TGGTACTGGCCAATCT TTCT CTG, and sequences of primers F-TGGACTTCGAGCAAGAGATG and R-GAAGGAAGGCTGGAAGAGTG for the β-actin cDNA amplification.

2.6. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) assay

The ERα and β-actin genes were measured by qRT-PCR device (Applied BioSystem A, Step One™, USA) according to the manufacturer's protocols. The reactions were performed on the basis of Syber Green-I (Maxima SYBR-Green/ROX qPCR MasterMix k-0221, ThermoScientific, Germany) with melting curve for both groups in the range of 60 to 95 °C to evaluate the specific sequences of genes along with their temperature cycles. The protocols of genes ERα and β-actin for qRT-PCR reaction were as follows, respectively: initial denaturation at 95 °C for 5 min (holding step), followed by 40 cycles of denaturation at 95 °C for 30 s (denaturation step), annealing at 57 °C for 40 s (annealing step), and extension at 95 °C for 15 s (extension step). Initial denaturation at 95 °C for 5 min (holding step), followed by 40 cycles of denaturation at 95 °C for 15 s (denaturation step), annealing at 60 °C for 15 s (annealing step), and extension at 72 °C for 20 s (extension step). The reaction was carried out in a final volume of 12 μl based on the desired amounts. To ensure for presence of DNA in the products of the qRT-PCR device, electrophoresis on a 1.2 % agarose gel in 0.5 (ng/μl) ethidium bromides was applied. Finally, the mean of threshold cycle (CT) data was calculated by the $2^{-\Delta\Delta CT}$ method [18].

2.7. Statistical analysis

Data analysis was evaluated by statistical software program SPSS (SPSS Co, Chicago IL, version 23) for windows. Statistical significance was considered at a $p < 0.05$ of two-tailed. All data are expressed as means ± standard deviation (SD) and checked for normality using Kolmogorov-Smirnov and homogenize of variances (Levene's test). To evaluate the difference between baseline- and week 12 values for each group paired samples *t*-test was employed. Analysis of Covariance (ANCOVA) test was applied to determine the changes of all variables during the time.

3. Results

3.1. Physiological characteristics

Baseline indices of OPMW did not show any significant differences between groups in physiological characteristics ($p > 0.05$, Table 1). After 12 weeks, METs value was significantly increased while TBF percentage was significantly decreased in the EX group ($p < 0.05$, Table 1). In addition, 17β-estradiol and progesterone were significantly decreased in the C group ($p < 0.05$, Table 1). The mean change TBF percentage was significantly decreased during time in the EX group compared to the C group ($p = 0.023$), whereas the mean change METs was significantly increased ($p = 0.001$). The effect size (Eta) coefficient showed 25.7 % decrease in TBF percentage and 97.9 % increase in METs in the EX group compared to the C group, whereas the other factors did not show considerable changes (Table 1).

3.2. Menopause status

Baseline values in menopause status parameters did not show significant differences in between-groups ($p > 0.05$, Table 1). After 12 weeks, 17β-estradiol and progesterone were significantly decreased in the C group ($p < 0.05$, Table 1). The mean changes in menopause status parameters did not show considerable changes during the time (Table 1).

3.3. Dietary intake

Dietary intake status in total energy intake (TEI), fat, carbohydrate (CHO), and protein did not show any significant within/between group differences as well as food intake during the time among OPMW ($p > 0.05$, Table 2).

3.4. Lymphocyte ERα gene expression

There was no significant difference in the lymphocyte ERα-mRNA expression at baseline ($p > 0.05$, Table 3). After 12 weeks of aerobic exercise intervention, lymphocyte ERα-mRNA expression was significantly increased in the EX group ($p < 0.05$, Table 3). The mean change during the time in the lymphocyte ERα-mRNA expression was significantly increased in the EX group compared to the C group ($p = 0.009$, Table 3). The Eta coefficient showed 28.2 % increase in the lymphocyte ERα-mRNA expression in the EX group compared to the C group (Table 3).

3.5. CRF parameters

Baseline values of CRF parameters did not show considerable differences in between-groups ($p > 0.05$, Table 3). After 12 weeks, VO_{2max} and walking-jogging time to exhaustion (WJTE) were significantly increased in the EX group ($p < 0.05$, Table 3). The mean changes VO_{2max} and WJTE were significantly increased during the time in the EX group compared to the C group ($p = 0.001$, Table 3). The Eta coefficient showed 53.4 % and 55.1 % increase in VO_{2max} and WJTE in the EX group compared to the C group, respectively (Table 3).

3.6. Echocardiographic parameters

Baseline values in echocardiographic parameters did not show any significant differences in between-groups ($p > 0.05$, Table 3). After 12 weeks, mitral E-wave deceleration time (E-wave D time) was significantly decreased while left ventricular ejection fraction (LVEF), cardiac output (Q), and cardiac index were significantly increased in the EX group as well as P-wave velocity time integral (PVTI) in the C group ($p < 0.05$, Table 3). The mean changes LVEF ($p = 0.001$), Q ($p = 0.036$), and cardiac index ($p = 0.030$) were significantly increased during the time in the EX group compared to the C group, whereas left ventricular end-systolic diameter (LVESD) ($p = 0.013$) and E-wave D time ($p = 0.007$) were significantly decreased (Table 3). The Eta coefficient showed 28.3 % and 32.1 % decrease in LVESD and E-wave D time and 53.6 %, 22.3 % and 22.5 % increase in LVEF, Q and cardiac index in the EX group compared to the C group, respectively (Table 3).

4. Discussion

Our study clearly showed that 12 weeks of moderate intensity aerobic exercise at 65 %–70 % HR_{max}Reserve provided cardio-protective effects via echocardiographic parameters and probably cellular mechanism of lymphocyte ERα gene expression among OPMW. In other words, our data revealed that CRF, LVEF, Q, cardiac index and as well as lymphocyte

Table 3
The lymphocyte ERα gene expression, CRF and cardiovascular (echocardiographic) parameters of OPMWs at baseline and week 12 in the EX and C groups.

Variables	EX (n = 14)	C (n = 13)	p[b] value	p[c] value	Eta coefficient (%)
Lymphocyte ERα gene expression (fold change)					
Baseline	0.17 ± 0.21	0.42 ± 0.39	0.070	0.009[‡]	0.282 (28.2 %)
Week 12	18.53 ± 18.14	0.40 ± 0.36	0.002[†]		
p[a] value	0.005*	0.283			
Cardiorespiratory fitness (CRF) parameters					
VO_2max (ml/kg/min)					
Baseline	29.05 ± 2.25	30.03 ± 5.74	0.713	0.001[‡]	0.534 (53.4 %)
Week 12	33.56 ± 2.13	29.26 ± 6.41	0.174		
p[a] value	0.001*	0.132			
Walking & jogging time to exhaustion (min)					
Baseline	9.93 ± 2.63	9.77 ± 2.79	0.895	0.001[‡]	0.551 (55.1 %)
Week 12	12.16 ± 1.95	9.62 ± 2.60	0.026[†]		
p[a] value	0.001*	0.470			
Cardiovascular (echocardiographic) parameters					
Left ventricular end-diastolic diameter (cm)					
Baseline	4.61 ± 0.58	4.91 ± 0.43	0.142	0.100	0.136 (13.6 %)
Week 12	4.50 ± 0.42	4.92 ± 0.46	0.049[†]		
p[a] value	0.651	0.630			
Left ventricular end-systolic diameter (cm)					
Baseline	3.22 ± 0.54	3.47 ± 0.38	0.329	0.013[‡]	0.283 (28.3 %)
Week 12	2.87 ± 0.41	3.73 ± 0.77	0.007[†]		
p[a] value	0.144	0.271			
Left ventricular septum diastolic diameter (mm)					
Baseline	7.20 ± 1.25	7.16 ± 1.25	0.537	0.840	0.002 (0.2 %)
Week 12	7.54 ± 1.46	7.49 ± 1.29	0.882		
p[a] value	0.578	0.249			
Left ventricular posterior wall diastolic diameter (cm)					
Baseline	7.05 ± 1.75	7.54 ± 1.86	0.794	0.589	0.016 (1.6 %)
Week 12	7.14 ± 1.59	7.48 ± 1.80	0.542		
p[a] value	0.887	0.370			
Aortic root diameter (cm)					
Baseline	2.29 ± 0.29	2.36 ± 0.33	0.698	0.168	0.098 (9.8 %)
Week 12	2.17 ± 0.16	2.37 ± 0.27	0.162		
p[a] value	0.276	0.832			
Left atrial area diameter (cm)					
Baseline	2.52 ± 0.34	2.51 ± 0.43	0.929	0.102	0.134 (13.4 %)
Week 12	2.40 ± 1.31	2.58 ± 0.46	0.190		
p[a] value	0.231	0.153			
Right ventricular diameter (cm)					
Baseline	2.21 ± 0.28	2.50 ± 0.35	0.051	0.556	0.019 (1.9 %)
Week 12	2.22 ± 0.25	2.38 ± 0.39	0.221		
p[a] value	0.913	0.066			
Right atrial area volume (cm^2)					
Baseline	10.58 ± 3.14	11.20 ± 3.79	0.681	0.261	0.066 (6.6 %)
Week 12	10.41 ± 2.23	11.70 ± 2.75	0.308		
p[a] value	0.830	0.614			
E velocity (cm/s)					
Baseline	0.68 ± 0.10	0.74 ± 0.18	0.354	0.458	0.029 (2.9 %)
Week 12	0.72 ± 0.11	0.80 ± 0.19	0.189		
p[a] value	0.106	0.209			
A velocity (cm/s)					
Baseline	0.61 ± 0.16	0.69 ± 0.11	0.218	0.227	0.076 (7.6 %)
Week 12	0.56 ± 0.11	0.64 ± 1.08	0.018[†]		
p[a] value	0.121	0.082			
Mitral E-wave deceleration time (ms)					
Baseline	190.58 ± 41.65	196.90 ± 62.35	0.780	0.007[‡]	0.321 (32.1 %)
Week 12	158.83 ± 29.46	197.90 ± 30.54	0.101[†]		
p[a] value	0.007*	0.965			
Aortic velocity time integral (cm)					
Baseline	17.00 ± 2.33	18.00 ± 3.26	0.290	0.199	0.088 (8.8 %)
Week 12	17.16 ± 2.55	19.00 ± 2.82	0.182		
p[a] value	0.504	0.191			
Pulmonary velocity time integral (cm)					
Baseline	17.41 ± 3.44	16.80 ± 2.34	0.637	0.402	0.037 (3.7 %)
Week 12	17.41 ± 2.46	17.40 ± 2.22	0.746		
p[a] value	1.00	0.024*			
Left ventricular ejection fraction (%)					
Baseline	55.00 ± 1.06	54.25 ± 1.20	0.137	0.001[‡]	0.536 (53.6 %)
Week 12	60.00 ± 2.88	54.25 ± 1.89	0.001[†]		
p[a] value	0.001*	1.00			
Cardiac output (l/min)					
Baseline	4.375 ± 0.48	4.169 ± 0.60	0.400	0.036[‡]	0.223 (22.3 %)
Week 12	5.041 ± 0.94	4.155 ± 0.67	0.024[†]		

Table 3 (*continued*)

Variables	EX (n = 14)	C (n = 13)	p^b value	p^c value	Eta coefficient (%)
p^a value	0.035*	0.876			
Cardiac index (l/min/m^2)					
Baseline	2.44 ± 0.29	2.30 ± 0.33	0.313	0.030‡	0.225 (22.5 %)
Week 12	2.88 ± 0.61	2.30 ± 0.35	0.016†		
p^a value	0.018*	0.986			

Note. EX = exercise group; C = control group; Eta = partial Eta squared or estimates of effect size; values are mean ± SD.

* $p^a < 0.05$, significantly different from baseline values by paired samples *t*-test (within groups, baseline vs. week 12).

† $p^b < 0.05$, significantly different baseline and also week 12 values by independent samples t-test (between groups, baseline EX vs. baseline C; and week12 EX vs. week 12 C).

‡ $p^c < 0.05$, significantly different between groups during the time (interaction) analyzed by univariate analysis of variance.

ERα-mRNA expression were increased with moderate intensity aerobic exercise in the EX group compared to the C group, whereas E-wave D time and LVESD were decreased.

Recent investigations on estrogen receptors report a cardio-protective role of ERα in both sexes after myocardial infarction (MI) in the heart of animal models [3,19,20]. In addition, recent studies reported that ERα-deficient in both male and female mice models is associated with obesity and insulin resistant [3,21]. Recently, a study reported that exercise-induced physiological myocardial hypertrophy is mediated by ERs in mice models [4]. These findings indicate the important role of ERs, especially ERα in cardio-protective function of animal models. Furthermore, a recent study reported that a higher expression of ERα-mRNA detected in the right atrial myocardium of human patients with congenital cardiac disease was associated with lower myocardial damage, improved lung function, lower water retention, and lower cytolysis in the early post-operative period might support the assumed cardio-protective role of ERs in this patient population [12]. In particular, it seems that the relationship between the expression of ER-mRNA and brain natriuretic peptide (BNP)-mRNA might indicate a role of hemodynamic overload in the upregulation of ERs as it has been shown in adult patients with aortic stenosis [12,22]. Recently, a study demonstrated ERα-mRNA expression detected in the left ventricular myocardial biopsies of postmenopausal women patients with aortic valve stenosis [22]. On the other hand, both ERα and ERβ are expressed in T-lymphocytes in animals as well as humans, which are associated with T-lymphocytes biology [23–26]. Thus, it is possible that lymphocyte ERα-mRNA may reflect cardiac tissue levels in OPMW. However, the molecular mechanism of cross-talk between the ERα signaling and T-lymphocyte (T-cell) responses following exercise interventions is unclear, and further studies are needed.

Our study for the first time showed that moderate intensity aerobic exercise at 65 %–70 % HR_{max}Reserve resulted in considerable increases in lymphocyte ERα gene expression after 12 weeks of aerobic exercise intervention among OPMW. To our knowledge, no studies have investigated lymphocyte ERα gene expression response to moderate intensity aerobic exercise in OPMW. The results of this study demonstrated that moderate intensity aerobic exercise was improved physiological adaptations of the heart including CRF, VO_{2max}, WJTE, LVESD, E-wave D time, LVEF, Q, and cardiac index in OPMW although the circulating levels of 17β-estradiol and progesterone remained unchanged. The results of our study were consistent with the results of some previous studies [4,27–30] and were inconsistent with others [31,32].

Dworatzek et al. reported that the exercise-induced physiological myocardial hypertrophy is mediated by ERs in mice models [4], which was consistent with the findings of the current study. In addition, Pandey et al. reported that short-term exercise training is associated with considerable improvement in CRF [27]. Our results were consistent with those studies with similar aspects to our independent variables, protocol and type of exercise, and gender including Mahmoodzadeh & Dworatzekand [3] and Dworatzek et al. [4] (ERα role in cardio-protective function and exercise-induced physiological myocardial hypertrophy, respectively), Tartibian et al. (12 weeks of walking-jogging aerobic exercise with 70 % HR_{max}Reserve, three sessions per week, and 60 min per training session in OPMW) [28] and Pandey et al. (the same exercise type of aerobic exercise intervention) [27]. In contrast, the difference in the type of exercise (resistance training) in Moghadasi & Siavashpour study [32], as well as the age [32] and health status [31] differences of participants in other studies, may be of possible inconsistency reasons with the results of our study.

The results of recent studies reported that exercise-induced ERα gene expression improves cardiac function and morphology through physiological myocardial hypertrophy-related signaling pathways including phosphoinositide 3-kinase (PI3-K)-serine/threonine kinase (AKT), glycogen synthase kinase-3β (GSK-3β), mitogen-activated protein kinase (MAPK), AMP-dependent protein kinase (AMPK), and p70 s6 kinase (S6k) and its down-stream target s6 ribosomal protein (rps6) in mice models [3,4]. Also, optimal reduction in the E-wave D time indicates a supernormal diastolic function. In other words, a vigorous recoil of the ventricle during diastole is demonstrated in young and physically active individuals [33], which was observed in the EX group compared to the C group. The results of our study demonstrated an increase in the flexibility and elasticity of the LV muscle in the EX group. Given these positive adaptations in diastolic and systolic function, it is possible that increased lymphocyte ERα-mRNA expression via aerobic exercise is associated with a supernormal diastolic function during menopause.

It is well-established that moderate intensity aerobic exercise increases CRF [27,28,34], cardio-protective effects [35], and quality of life in humans [36]. In other words, aerobic exercise-induced CVS adaptations can be accompanied by a better quality of life and decreased CVD in sedentary OPMW [1,28]. In addition, moderate intensity aerobic exercise provides changes in the heart that contribute to the greater peak Q and aerobic capacity [35,37]. Changes in LVEF and blood volume play the primary role in enhancing stroke volume (SV), and hence Q and cardiac index [37], which were observed in the EX group compared to the C group of our study. Furthermore, cardiovascular adaptations to aerobic exercise training allow the heart to maintain a given submaximal work level at a lower heart rate, and thus with less strain on the heart [37]. It is possible that moderate intensity aerobic exercise in sedentary OPMW increases maximal exercise performance reflecting an increase in VO_{2max}. In this regard, the results of studies revealed that the age, gender differences, fitness level, aerobic capacity, type of exercise, and duration of the physically inactive can be involved in CVS changes in older adults [37].

Our study demonstrated that 12 weeks of aerobic exercise intervention improved CRF through positive adaptations in physiological indices including an increase in the VO_{2max}, WJTE, METs, and TBF percentage in OPMW while other variables did not show considerable changes during the time. CRF as a genetic factor is similar in importance to other major physiological risk factors for CVD, but it is also highly adaptable via aerobic exercise [33,34,37,38]. The results of studies well-established that moderate intensity aerobic exercise is one of the non-pharmacological interventions to improve CRF [28,34]. Also, the recent studies have reported that increased CRF is associated with reduced risk factors of CVD and lower all-cause and cardiovascular mortality [9,27,28] which can promote healthy cognitive and psychosocial function in OPMW [39]. It seems that increased CRF and decreased TBF lead to a reduction in aging-induced diseases such as CVD [40], sarcopenia, and sarcopenia obesity in older adults [41,

42]. In other words, it is possible that increased efficiency of CVS and decreased TBF can delay aging-induced risk factors such as CVD and sarcopenia obesity during menopause although the mechanism is unclear [39].

Recent studies reported that menopause status is associated with changes in body composition and physiological parameters such as increased TBF and central adiposity [43,44], decreased fat-free mass and muscular strength [43], decreased energy expenditure [44,45], decreased CRF as well as decreased sex hormones [31]. It is well-established that serum levels of sex hormones such as 17β-estradiol and progesterone decrease significantly due to both physiological and psychological changes such as loss of skeletal muscle mass, decreased CRF and poor quality of life during menopause [31,46]. Furthermore, aromatization of androgens is strongly affected by aging, as specific activity of aromatase increases with menopause and aging [47]. In contrast, reduced central adiposity and total body fat [48], increased VO_{2max} as well as the maintenance of sex hormones of 17β-estradiol and progesterone by aerobic exercise intervention can be considered as aerobic exercise-induced positive hormonal and physiological adaptations and improved CRF during menopause [49], which were observed in the EX group compared to the C group of this study. In other words, potential mechanisms related to the maintenance of sex hormones by aerobic exercise during menopause may be the muscle mass [43], central adiposity and fat mass [43,44], glucose and lipid metabolism [50], menopause age or reproductive function [17], mitochondrial function [50] as well as changes in 17β-estradiol and progesterone receptors in OPMW [17]. However, the potential mechanisms of exercise intervention-related sex hormone changes are unknown and require further studies. The results of studies demonstrated that the aging-induced CVS changes are associated with changes in HR_{max}, contractility, end-diastolic volume (EDV), end-systolic volume (ESV), SV, prolonged diastolic relaxation, prolonged systolic contraction, and sympathetic signaling during menopause. As mentioned above, increase in the E-wave D time is associated with diastolic dysfunction and aging process in the heart during menopause, which was observed in the C group.

4.1. Limitations

The current study had some limitations. We only measured ERα gene expression at the mRNA level, but not at the protein level. In addition, the human volunteers as participants in our study were only sedentary OPMW aged 45–65 years and consequently the findings of our study can't be generalized to the other statistical populations such as young women, girls, men, and boys.

5. Conclusions

In conclusion, moderate intensity aerobic exercise at 65 %–70 % HR_{max}Reserve was effective in enhancing CRF, cardiac systolic function, cardiac diastolic function, and cardio-protective effects as well as increasing cardiac index and ERα gene expression in sedentary OPMW. In other words, aerobic exercise for 12 weeks in OPMW can increase exercise capacity and lead to an associated improvement in left ventricular cardiac functional, enhanced ERα gene expression and preservation of estrogen levels during menopause. However, further studies are needed to determine the effect of aerobic exercise on cardiac function, CRF and gene expression of ERs in sedentary OPMW. In addition, the effects of types of exercise interventions on the cross-talk between the ERα signaling and lymphocyte responses during menopause or CVD could be investigated in future studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to the OPMW who participated in this study.

References

[1] Azadpour N, Tartibian B, Koşar ŞN. Effects of aerobic exercise training on ACE and ADRB2 gene expression, plasma angiotensin II level, and flow-mediated dilation: a study on obese postmenopausal women with prehypertension. Menopause. 2016;24(3):269–77. https://doi.org/10.1097/GME.0000000000000762.
[2] Garcia M, Mulvagh SL, Merz CN, Buring JE, Manson JE. Cardiovascular disease in women: clinical perspectives. Circ Res. 2016;118(8):1273–93. https://doi.org/10.1161/CIRCRESAHA.
[3] Mahmoodzadeh S, Dworatzek E. The role of 17β-estradiol and estrogen receptors in regulation of Ca2+ channels and mitochondrial function in cardiomyocytes. Front Endocrinol. 2019;10:310. https://doi.org/10.3389/fendo.2019.00310.
[4] Dworatzek E, Mahmoodzadeh S, Schubert C, Westphal C, Leber J, Kusch A, et al. Sex differences in exercise-induced physiological myocardial hypertrophy are modulated by oestrogen receptor beta. Cardiovasc Res. 2014;102(3):418–28. https://doi.org/10.1093/cvr/cvu065.
[5] Mahmoodzadeh S, Eder S, Nordmeyer J, Ehler E, Huber O, Martus P, et al. Estrogen receptor alpha up-regulation and redistribution in human heart failure. FASEB J. 2006;20:926–34. https://doi.org/10.1096/fj.05-5148com.
[6] Ropero AB, Eghbali M, Minosyan TY, Tang G, Toro L, Stefani E. Heart estrogen receptor alpha: distinct membrane and nuclear distribution patterns and regulation by estrogen. J Mol Cell Cardiol. 2006;41(3):496–510. https://doi.org/10.1016/j.yjmcc.2006.05.022.
[7] Barros RP, Gustafsson JÅ. Estrogen receptors and the metabolic network. Cell Metab. 2011;14(3):289–99. https://doi.org/10.1016/j.cmet.2011.08.005.
[8] Karinkanta S. Exercise: a comprehensive tool for ageing women to take care of their health. Menopause. 2017;24(1):7–8. https://doi.org/10.1097/GME.0000000000000799.
[9] Tartibian B, Botelho Teixeira AM, Baghaiee B. Moderate intensity exercise is associated with decreased angiotensin-converting enzyme, increased β2-adrenergic receptor gene expression, and lower blood pressure in middle-aged men. J Aging Phys Act. 2015;23(2):212–20. https://doi.org/10.1123/japa.2013-0136.
[10] Tartibian B, Hajizadeh Maleki B, Kanaley J, Sadeghi K. Long-term aerobic exercise and omega-3 supplementation modulate osteoporosis through inflammatory mechanisms in post-menopausal women: a randomized, repeated measures study. Nutr Metab (Lond). 2011;8(1):71. https://doi.org/10.1186/1743-7075-8-71.
[11] Oláh A, Mátyás C, Kellermayer D, Ruppert M, Barta BA, Sayour AA, et al. Sex differences in morphological and functional aspects of exercise-induced cardiac hypertrophy in a rat model. Front Physiol. 2019;10:889. https://doi.org/10.3389/fphys.2019.00889.
[12] Rouatbi H, Farhat N, Heying R, Vazquez-Jimenez JF, Parent AS, Seghaye MC. Myocardial expression of estrogen receptor-mRNA is associated with lower markers of post-operative organ damage in young patients with congenital cardiac defect. Front Pediatr. 2021;9:729198. https://doi.org/10.3389/fped.2021.729198.
[13] Malandish A, Tartibian B, Sheikhlou Z, Afsargharehbagh R, Rahmati M. The effects of short-term moderate intensity aerobic exercise and long-term detraining on electrocardiogram indices and cardiac biomarkers in postmenopausal women. J Electrocardiol. 2020;60:15–22. https://doi.org/10.1016/j.jelectrocard.2020.03.004.
[14] Mosteller RD. Simplified calculation of body surface area. N Engl J Med. 1987;317(17):1098.
[15] Bailey RL, Miller PE, Mitchell DC, Hartman TJ, Lawrence FR, Sempos CT, et al. Dietary screening tool identifies nutritional risk in older adults. Am J Clin Nutr. 2009;90(1):177–83. https://doi.org/10.3945/ajcn.2008.27268.
[16] Saremi MA, Saremi M, Tavallaei M. Rapid genomic DNA extraction (RGDE). Forensic Sci Int Genet Suppl S. 2008;1(1):63–5. https://doi.org/10.1016/j.fsigss.2007.12.001.
[17] Malandish A, Tartibian B, Rahmati M, Afsargharehbagh R, Sheikhlou Z. The effect of moderate-intensity aerobic training on pulmonary function and estrogen receptor-alpha gene in postmenopausal women with vitamin D deficiency: a randomized control trial. Respir Physiol Neurobiol. 2020;281:1–8. https://doi.org/10.1016/j.resp.2020.103510.
[18] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25(4):402–8. https://doi.org/10.1006/meth.2001.1262.
[19] Wang M, Crisostomo P, Wairiuko GM, Meldrum DR. Estrogen receptor-alpha mediates acute myocardial protection in females. Am J Physiol Heart Circ Physiol. 2006;290(6):H2204–9. https://doi.org/10.1152/ajpheart.01219.2005.
[20] Zhai P, Eurell TE, Cooke PS, Lubahn DB, Gross DR. Myocardial ischemia-reperfusion injury in estrogen receptor-alpha knockout and wild-type mice. Am J Physiol Heart Circ Physiol. 2000;278(5):H1640–7. https://doi.org/10.1152/ajpheart.2000.278.5.H1640.
[21] Allard C, Morford JJ, Xu B, Salwen B, Xu W, Desmoulins L, et al. Loss of nuclear and membrane estrogen receptor-α differentially impairs insulin secretion and action in male and female mice. Diabetes. 2019;68(3):490–501. https://doi.org/10.2337/db18-0293.
[22] Nordmeyer J, Eder S, Mahmoodzadeh S, Martus P, Fielitz J, Bass J, et al. Upregulation of myocardial estrogen receptors in human aortic stenosis. Circulation. 2004;110:3270–5. https://doi.org/10.1161/01.CIR.0000147610.41984.E8.
[23] Rosenzweig R, Gupta S, Kumar V, Gumina RJ, Bansal SS. Estrogenic bias in T-lymphocyte biology: implications for cardiovascular disease. Pharmacol Res. 2021;170:105606. https://doi.org/10.1016/j.phrs.2021.105606.
[24] Rosenzweig R, Kumar V, Gupta S, Bermeo-Blanco O, Stratton MS, Gumina RJ, et al. Estrogen receptor-β agonists modulate T-lymphocyte activation and ameliorate left ventricular remodeling during chronic heart failure. Circ Heart Fail. 2022;15(7):e008997. https://doi.org/10.1161/CIRCHEARTFAILURE.121.008997.
[25] Gustafsson KL, Nilsson KH, Farman HH, Andersson A, Lionikaite V, Henning P, et al. ERα expression in T lymphocytes is dispensable for estrogenic effects in bone. J Endocrinol. 2018;238(2):129–36. https://doi.org/10.1530/JOE-18-0183.
[26] Dayoub O, Le Lay S, Soleti R, Clere N, Hilairet G, Dubois S, et al. Estrogen receptor α/HDAC/NFAT axis for delphinidin effects on proliferation and differentiation of T

lymphocytes from patients with cardiovascular risks. Sci Rep. 2017;7(1):9378. https://doi.org/10.1038/s41598-017-09933-4.
[27] Pandey A, Johnson JL, Slentz CA, Ross LM, Agusala V, Berry JD, et al. Short-term changes in cardiorespiratory fitness in response to exercise training and the association with long-term cardiorespiratory fitness decline: the STRRIDE Reunion study. J Am Heart Assoc. 2019;8:e012876.
[28] Tartibian B, Malandish A, Afsargharehbagh R, Eslami R, Sheikhlou Z. Assessment of hepatic and lipid profiles following 12 weeks of aerobic exercise in overweight postmenopausal women. Int J Basic Sci Med. 2018;3(4):159–67. https://doi.org/10.15171/ijbms.2018.28.
[29] Mandsager K, Harb S, Cremer P, Phelan D, Nissen S, Jaber W. Association of cardiorespiratory fitness with long-term mortality among adults undergoing exercise treadmill testing. JAMA Netw Open. 2018;1(6):e183605.
[30] Church TS, Earnest CP, Skinner JS, Blair SN. Effects of different doses of physical activity on cardiorespiratory fitness among sedentary, overweight or obese postmenopausal women with elevated blood pressure: a randomized controlled trial. JAMA. 2007;297(19):2081–91.
[31] Razzak AZ, Aamir Khan A, Imran Farooqui S. Effect of aerobic and anaerobic exercise on estrogen level, fat mass, and muscle mass among postmenopausal osteoporotic females. Int J Health Sci (Qassim). 2019;13(4):10–6. PMID: 31341450.
[32] Moghadasi M, Siavashpour S. The effect of 12 weeks of resistance training on hormones of bone formation in young sedentary women. Eur J Appl Physiol. 2013;113(1):25–32. https://doi.org/10.1007/s00421-012-2410-0.
[33] Claessens PJ, Claessens CW, Claessens MM, Claessens MC, Claessens JE. Supernormal left ventricular diastolic function in triathletes. Tex Heart Inst J. 2001;28(2):102–10. PMID:11453120.
[34] Hajizadeh Maleki B, Tartibian B, Mooren FC, FitzGerald LZ, Krüger K, Chehrazi M, et al. Low-to-moderate intensity aerobic exercise training modulates irritable bowel syndrome through antioxidative and inflammatory mechanisms in women: results of a randomized controlled trial. Cytokine. 2018;102:18–25. https://doi.org/10.1016/j.cyto.2017.12.016.
[35] Rognmo Ø, Moholdt T, Bakken H, Hole T, Mølstad P, Myhr NE, et al. Cardiovascular risk of high- versus moderate-intensity aerobic exercise in coronary heart disease patients. Circulation. 2012;126(12):1436–40. https://doi.org/10.1161/CIRCULATIONAHA.112.123117.
[36] Myers VH, McVay MA, Brashear MM, Johannsen NM, Swift DL, Kramer K, et al. Exercise training and quality of life in individuals with type 2 diabetes: a randomized controlled trial. Diabetes Care. 2013;36(7):1884–90. https://doi.org/10.2337/dc12-1153.
[37] Tartibian B, Afsargharehbagh R, Malandish A, Sheikhlou Z. Assessment of electrocardiogram indices in postmenopausal women: effects of aerobic exercise and detraining. Int J Basic Sci Med. 2018;3(1):38–45. https://doi.org/10.15171/ijbms.2018.07.
[38] Stamatakis E, Koster A, Mork PJ. Cardiorespiratory fitness and long-term mortality: targeting the least fit with incidental physical activity. J Am Coll Cardiol. 2018;72(9):996–8. https://doi.org/10.1016/j.jacc.2018.07.001.
[39] McKinney J, Lithwick DJ, Morrison BN, Nazzari H, Isserow S, Heilbron B, et al. The health benefits of physical activity and cardiorespiratory fitness. BC Med J. 2016;58(3):131–7.
[40] Malandish A, Ghadamyari N, Karimi A, Naderi M. The role of exercise training on cardiovascular peptides in patients with heart failure: a systematic review and meta-analysis. Curr Res Physiol. 2022;5:270–86. https://doi.org/10.1016/j.crphys.2022.06.004.
[41] Stephen WC, Janssen I. Sarcopenic-obesity and cardiovascular disease risk in the elderly. J Nutr Health Aging. 2009;13(5):460–6.
[42] Lee I, Cho J, Jin Y, Ha C, Kim T, Kang H. Body fat and physical activity modulate the association between sarcopenia and osteoporosis in elderly korean women. J Sports Sci Med. 2016;15(3):477–82. PMID: 27803626.
[43] Messier V, Rabasa-Lhoret R, Barbat-Artigas S, Elisha B, Karelis AD, Aubertin-Leheudre M. Menopause and sarcopenia: a potential role for sex hormones. Maturitas. 2011;68(4):331–6. https://doi.org/10.1016/j.maturitas.2011.01.014.
[44] Greendale GA, Sternfeld B, Huang M, Han W, Karvonen-Gutierrez C, Ruppert K, et al. Changes in body composition and weight during the menopause transition. JCI Insight. 2019;4(5):1–14. https://doi.org/10.1172/jci.insight.124865.
[45] Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. Endocr Rev. 2013;34(3):309–38. https://doi.org/10.1210/er.2012-1055.
[46] Blumel JE, Castelo-Branco C, Binfa L, Gramegna G, Tacla X, Aracena B, et al. Quality of life after the menopause: a population study. Maturitas. 2000;34(1):17–23.
[47] Hale GE, Robertson DM, Burger HG. The perimenopausal woman: endocrinology and management. J Steroid Biochem Mol Biol. 2014;142:121–31. https://doi.org/10.1016/j.jsbmb.2013.08.015.
[48] Khalafi M, Malandish A, Rosenkranz SK, Ravasi AA. Effect of resistance training with and without caloric restriction on visceral fat: a systemic review and meta-analysis. Obes Rev. 2021;22(9):e13275. https://doi.org/10.1111/obr.13275.
[49] Khalafi M, Malandish A, Rosenkranz SK. The impact of exercise training on inflammatory markers in postmenopausal women: a systemic review and meta-analysis. Exp Gerontol. 2021;150:111398. https://doi.org/10.1016/j.exger.2021.111398.
[50] Cho EJ, Choi Y, Jung SJ, Kwak HB. Role of exercise in estrogen deficiency-induced sarcopenia. J Exerc Rehabil. 2022;18(1):2–9. https://doi.org/10.12965/jer.2244004.002.