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Effects of brisk walking with or without music on body composition, standing balance, cardiovascular parameters, and salivary biomarkers in older women

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This study aimed to assess and compare changes in body composition, standing balance, cardiovascular parameters, and salivary biomarkers, particularly salivary antioxidant status, after brisk walking training with or without music in older women. Twenty-four subjects were randomly assigned to brisk walking groups: with music (BWM) (n=12) or without music (BW) (n=12). Eighteen subjects completed the exercise training (9 in each group), and their data were used for analysis. The research protocols were classified into three phases: pretraining phase, training phase, and posttraining phase, while the data collection was divided into four sessions: resting condition, during treadmill exercise testing, immediately posttreadmill exercise testing, and 5-min posttreadmill exercise testing defined as after the cool-down session. The results showed that 8 weeks of home-based brisk walking with or without music did not improve standing balance, blood pressure, salivary biomarkers including total protein concentration, and antioxidant status but maintained or prevented the decline of these parameters. Only the BWM group reduced fat mass relative to increasing fat-free mass ($P<0.05$) and improved recovery heart rate ($P<0.05$) by modifying cardiac autonomic control in posttreadmill exercise testing. Therefore, brisk walking with preferred music can be a tool to delay the progression of cardiovascular dysfunction in older women. A longer duration of the exercise program and larger groups of participants are needed for further investigation of brisk walking with or without music on physiological and biochemical changes.

Keywords: Brisk walking, Music, Older women, Cardiovascular system, Salivary biomarkers, Body composition

INTRODUCTION

It is well-known that the physiological functions of multiple organs progressively decline with age (Guo et al., 2022). These changes involve cellular and molecular pathogenesis, including a decrease in muscle contractile properties, the neurodegenerative process, oxidative damage, immune response and inflammatory processes, metabolic disturbance, and mitochondrial dysfunction. Previous studies have reported that older women have a higher incidence of Alzheimer disease, hypertension, heart disease, chronic obstructive pulmonary disease, along with a reduction in muscle strength compared to men (Hagg and Jylhava, 2021). Consequently, lifestyle modifications such as dietary changes and increased physical activity are crucial for delaying the onset and progression of these conditions in elderly women (Mattioli et al., 2022).

To date, exercise training is one of the powerful strategies to delay or prevent the progression of age-related diseases (Ciolac, 2013; Gronek et al., 2021). Walking is a simple exercise that is suitable for older adults. A previous study has revealed strong evidence of the benefits of brisk walking training in improving body compo-

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sition, muscle strength, and cardiorespiratory fitness in this population, while its effects on muscle endurance, flexibility, and balance remain limited (Bai et al., 2021). Nonetheless, it is still challenging to enhance the physical activity adherence in older adults (Collado-Mateo et al., 2021). Previously, listening to music during exercise has demonstrated improvements in both physical and mental health through psychological and physiological mechanisms (Ballmann, 2021; Terry et al., 2020). For instance, music can have positive effects on emotions and mood, leading to lower levels of stress, anxiety, and depression, and improving positive energy and vitality. It can also modulate physiological responses during exercise based on music tempo. A slow music tempo can preserve parasympathetic activity, reducing physical stress, while a fast music tempo can increase catecholamine release, improving muscle activation and metabolic responses. Additionally, music can increase the neuromuscular fatigue threshold, thereby enhancing exercise performance. Furthermore, a fast music tempo can increase cardiac output and oxygen consumption while regulating metabolic and hormonal responses, including cortisol bioavailability and lactate clearance during exercise. Recently, walking synchronized with music tempo has shown additional effects on health-related outcomes, such as the reduction in body fat ratio after 12 weeks of walking training, in pre-older sedentary females compared to walking without music (Wang et al., 2022). Moreover, brisk walking with music has a beneficial effect on sleep quality in older adults with insomnia (Huang et al., 2016). Thus, music may be used as a tool to maintain motivation, reduce fatigue perception, and enhance physical performance during exercise. However, there are limited studies comparing the physiological effects, particularly cardiovascular and autonomic control, of brisk walking training with or without music in older women.

In addition to age-related physiological changes, several biomarkers implicated in the aging process have been investigated (Engelfriet et al., 2013). These biomarkers include the DNA repair marker 8-hydroxydeoxyguanosine, metabolic and stress markers such as growth hormone, insulin-like growth factor 1, and cortisol, as well as inflammatory markers like C-reactive protein, interleukin-6, and tumor necrosis factor α. Furthermore, oxidative stress markers such as glutathione (GSH), superoxide dismutase (SOD), and nitric oxide have also been studied. Oxidative stress, defined as the imbalance between oxidants and antioxidants leading to oxidative damage, is known to be involved in the onset of several degenerative diseases in the elderly (Simioni et al., 2018). A recent study found that both regular continuous walking and intermittent walking can potentially reduce oxidative stress in hypertensive older adults by increasing blood GSH and decreasing serum malondialdehyde, a lipid peroxidation marker (Prasertsri et al., 2022). Furthermore, some evidence suggests that changes in salivary oxidative stress markers are comparable to plasma profiles and associated with exercise intensity (Alves et al., 2022; Souza et al., 2019). The noninvasive monitoring of salivary redox homeostasis in response and adaptation to exercise in older adults is of interest. Nevertheless, the investigation of salivary oxidant/antioxidant status after brisk walking training with or without music in older women remains unknown.

This study aimed to assess and compare the changes in the body composition, standing balance, cardiovascular parameters, and salivary biomarkers, with a particular focus on salivary antioxidant status, following brisk walking training with or without music among older women.

MATERIALS AND METHODS

Participants

This study received approval from the human research ethics committee of Chulabhorn Royal Academy (project code: EC019/2565), following the principles of the Declaration of Helsinki. The participants were enrolled via an electronic poster and QR code that provided information regarding the research's inclusion and exclusion criteria, the exercise protocol, and important details approved by the human research ethics committee. The inclusion criteria were older women with a body mass index (BMI) of 18.5–24.9 kg/m^2 and age of 60–75 years who performed exercises of moderate intensity for less than 150 min/wk. The exclusion criteria were individuals with musculoskeletal problems or underlying diseases that affected brisk walking ability, subjects who have experienced a severe acute respiratory syndrome coronavirus 2 infection within the past year, oral inflammation, current smoking status, regular intake of antioxidant supplements, uncontrolled hypertension, and diabetes. Initially, 53 older women were enrolled for eligibility assessment. Subsequently, 29 participants did not meet project criteria were not included. Before collecting data, participants were given the participant information sheet and consented in writing to participate in the study. The remaining 24 subjects were randomly assigned to either brisk walking without music (n = 12) or with music (n = 12). Finally, 18 subjects successfully completed the exercise training, with 9 in the brisk walking with music group and 9 in the brisk walking without music group. The data from these groups were utilized for the final analysis (Fig. 1). The sample size was determined using G*Power software

(Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) based on heart rate (HR) responses during treadmill exercise testing, and a sample size of n = 9 is considered sufficient to interpret the data.

Study and exercise protocols

In this study, research protocols were classified into three phases: pretraining phase, training phase, and posttraining phase (Fig. 1). During the pretraining phase, baseline data, including assessments of body composition, standing balance, cardiovascular parameters, and salivary samples, were collected from the participants. The data collection was divided into four sessions, depending on the variables being measured: resting condition, during treadmill exercise testing, immediately posttreadmill exercise testing, and 5-min posttreadmill exercise testing defined as after the cool-down session (Fig. 2). The treadmill exercise testing involved brisk walking without music at a moderate intensity (40%–59% of heart rate reserve [HRR]) for a duration of 25 min, including a warm-up period (5 min) to reach the target HR, the exercise testing period (20 min), and a cool-down period consisting of slow walking at 3 km/hr for 5 min at the end of the exercise session.

In the training phase, participants received an 8-week home-based exercise program. This program involved outdoor brisk walking at moderate intensity (40%–59% HRR) with or without music in the village. Participants were also instructed on how to monitor their HR, which could be estimated using a smartwatch provided by our project (XMSHS05HM, Anhui Huami Information Technology Co., Ltd., Anhui, China) or their self-provided device. The brisk walking training sessions lasted 30 min each and were conducted 3 days a week for the initial 4 weeks. Subsequently, the

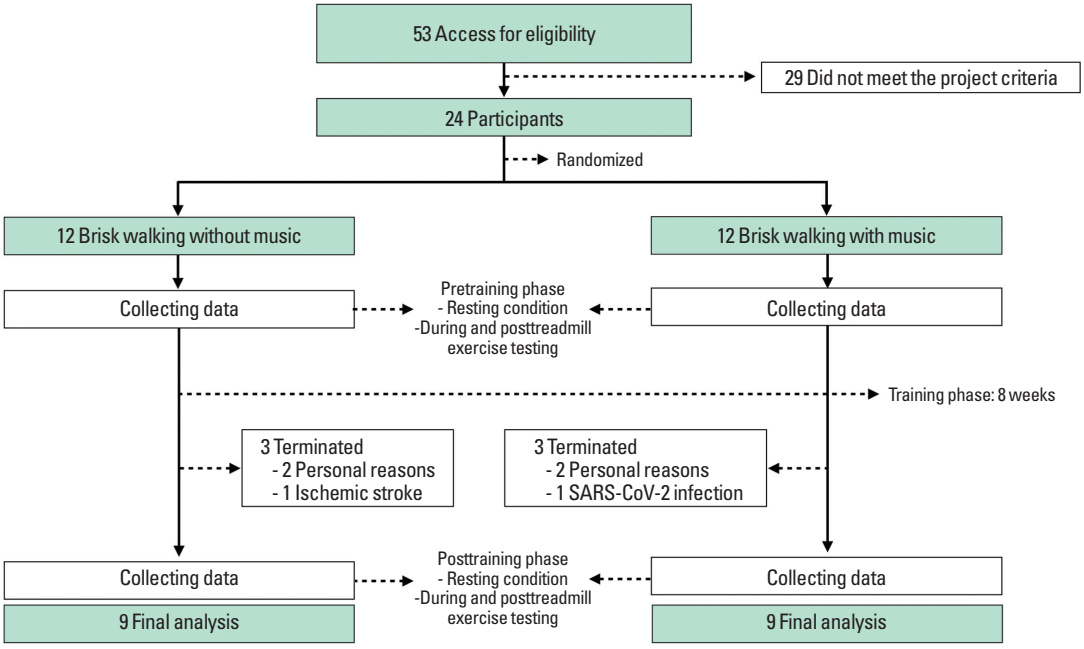

Fig. 1. The study design and protocol of this study.

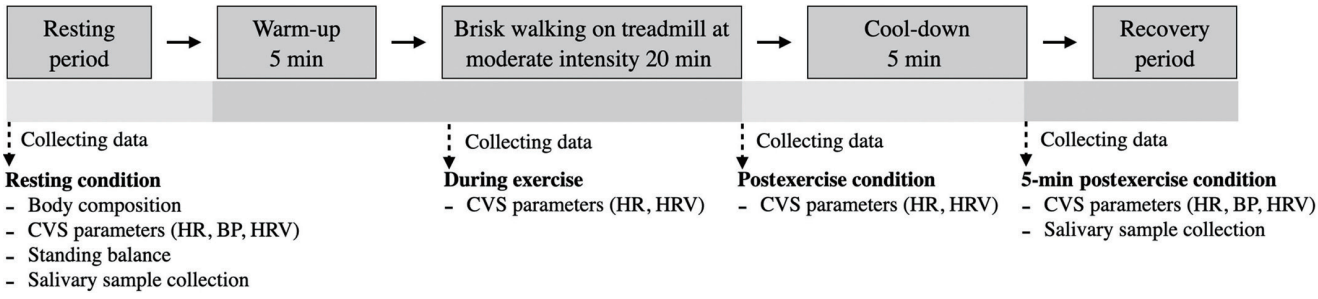

Fig. 2. Data collection at different time periods of treadmill exercise testing. CVS, cardiovascular system; HR, heart rate; BP, blood pressure; HRV, heart rate variability.

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exercise intensity was adjusted by increasing the frequency of brisk walking to 5 days a week for the following 4 weeks. Participants' self-exercise logbook and telecommunication through social networks were used to track and maintain participants' engagement with the exercise program. During the posttraining phase, data collection was repeated, mirroring the assessments conducted in the pretraining phase.

Music tempo and song selections

Music tempo in this study was determined using application GarageBand (Apple Inc., Cupertino, CA, USA). For this study, we selected the music tempo range of 109–132 beats per minute (bpm) based on evidence from a study on music tempo and exercise performance in resistance training (Ballmann et al., 2021). The preferred song playlist, a combination of Thai folk and country music styles, consists of 10 songs. This playlist was played on participants' phones at a comfortable speaker volume during brisk walking with music.

Measurement variables

In this study, variables related to body composition, standing balance, cardiovascular status, salivary flow rate, salivary pH, and salivary biomarkers were assessed. As mentioned earlier, a reduction in body fat ratio after 12 weeks was observed in walking training with music in pre-older sedentary females compared with walking training without music (Wang et al., 2022). We assessed body composition, including measurements of fat mass, fat-free mass, skeletal muscle mass, and total body water, to compare these changes in older women. We also investigated standing balance by measuring the sway velocity index (SVI), which reflects postural control, in both a comfortable stance and a narrow stance; these aspects among older adults trained with brisk walking still require further investigation (Bai et al., 2021). Moreover, cardiovascular parameters including HR, blood pressure, and HR variability (HRV) are incorporated in this study to provide insight into the information on brisk walking with or without music on cardiovascular health in older women, as we know that music can modulate psychological and physiological responses during exercise (Ballmann, 2021; Terry et al., 2020). We further investigate biochemical changes in salivary samples to provide information on noninvasive biomarkers linked to redox status and exercise (Jinakote et al., 2023; Souza et al., 2019) by measuring salivary flow rate, salivary pH, total protein concentration, and antioxidant level/activity including total antioxidant capacity (TAC), reduced GSH, catalase (CAT), and SOD in older women.

Measurement of the body composition

At the first visit of pretraining phase and after the completion of the 8-week training sessions, participants' data, which included measurements of fat mass, fat-free mass, skeletal muscle mass, and total body water, were obtained using the Seca medical Body Composition Analyzer (5141321004, Seca GmbH & Co., KG, Hamburg, Germany). Additionally, weight, height, and BMI were also recorded for subsequent analysis.

Measurement of the standing balance

The participants' standing balance parameter, SVI, a derived value from velocity and patient height, was obtained for further analysis. It was measured while they performed a comfortable stance and a narrow stance (feet closer together) with eyes open for 30 sec using the Biodex Balance System SD (Biodex Medical Systems, Inc., Shirley, NY, USA).

Measurement of the cardiovascular variables

The HR, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were determined in both the pretreadmill and 5-min posttreadmill exercise testing during the pretraining and posttraining periods using an automated sphygmomanometer (2130, SunTech Medical, Inc., Morrisville, NC, USA). Mean arterial pressure (MAP) was calculated as DBP+1/3 (SBP–DBP). Cardiac autonomic regulation was assessed through HRV. Resting HRV was recorded for 5 min as participants sat in a relaxed posture on a comfortable chair. The Polar H10 (Polar Electro Oy, Kempele, Finland) synchronized with the Elite HRV application installed on an iPad (MUUK2TH/A, Apple Inc., Cupertino, CA, USA) was used in this study. Subsequently, RR interval data were exported from the Elite HRV application, and Kubios HRV software (Kubios Oy, Kuopio, Finland) was utilized for analysis, as previously reported (Jinakote et al., 2023). Additionally, RR intervals were recorded during the entire treadmill exercise and 5-min post-cool-down session (5-min postexercise condition). All data were exported and further analyzed as described above.

Salivary collection, preparation, and storage

Participants were instructed to collect unstimulated salivary samples by spitting into 50-mL conical sterile polypropylene centrifuge tubes for 10 min. This data collection took place during both resting conditions and 5-min posttreadmill exercise testing at the first visit of the pretraining phase and after completing the training sessions. After measuring salivary volume and salivary pH, each sample was transferred to a sterile 1.5-mL microcentri-

fuge tube and then centrifuged using a refrigerated centrifuge (TGL-16, UGAIYA Bio-Sciences Co., Ltd., Osaka, Japan) at 5,000×*g*, 4°C, for 40 min. The clear supernatant of each sample was then transferred to other tubes, and a protease inhibitor cocktail (HY-K0010, Medchemexpress LLC, Monmouth Junction, NJ, USA) was added to preserve the protein and prevent degradation. These samples were subsequently stored at -20°C and were analyzed within 4 months.

Measurement of the salivary flow rate and salivary pH

The previously mentioned 50-mL conical sterile polypropylene centrifuge tubes were weighed both before and after collecting saliva in order to determine the salivary volume using analytical balance (ATX224R, Shimadzu, Kyoto, Japan), assuming a salivary density of 1 g/mL. The salivary flow rate was computed and expressed in mL/min. Salivary pH was determined using a pH meter.

Chemicals

Bradford reagent (#5000006) and bovine serum albumin (BSA) were purchased from Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, CA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), reduced GSH, CAT from bovine liver, Trolox, and *o*-phthalaldehyde were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals with high purity were obtained from various commercial sources.

Measurement of the salivary biomarkers

On the day of the experiments, the samples were thawed, centrifuged at 5,000×*g* and 4°C for 10 min, and the clear supernatant in each sample was transferred to new microcentrifuge tubes for further analysis. The salivary total protein concentration, which serves as a biomarker of exercise intensity (Souza et al., 2019), was quantified by a colorimetric assay using Bradford reagent, following the manufacturer's instructions as previously described (Jinakote et al., 2023). The results were expressed as mg/mL using the standard curve with BSA. The salivary TAC was determined by modifying the DPPH radical scavenging assay reported in a previous study (Shimamura et al., 2014). The 0.2 mM DPPH was dissolved in absolute ethanol and protected from light for 2 hr at room temperature prior to use. A 20 µL of the diluted sample and 80 µL of 0.1 M Tris-HCl buffer (pH, 7.4) were added into a 96-well clear plate. Then 100 µL of DPPH was added to each well before incubation in the dark at room temperature for 30 min. The absorbance in each well was subsequently read at a wavelength of 517 nm using a hybrid multimode microplate reader (Synergy H1MF, BioTek Instruments Inc., Winooski, VT, USA). The results were represented as µM standard Trolox, a potent antioxidant, equivalent.

The concentration of salivary GSH, which plays an important role in redox homeostasis and the antioxidant defense system during exercise (Kerksick and Willoughby, 2005), was measured using a previously reported method (Souza et al., 2019). The salivary samples were mixed with an equal volume of 5% w/v metaphosphoric acid into microcentrifuge tubes and then centrifuged using a refrigerated centrifuge at 7,000×*g*, 4°C, for 10 min. Then 30 µL of supernatant was transferred into a 96-well black plate. In addition, 185 µL of 100 mM sodium phosphate buffer (pH, 8.0) containing 5 mM ethylenediaminetetraacetic acid (EDTA) was added into each well. The last step comprised the addition of 15 µL of *o*-phthalaldehyde (1 mg/mL dissolved in absolute methanol). The plate was incubated at room temperature in the dark for 15 min. The fluorescent intensity was detected at an excitation/emission wavelength of 350/420 nm using a hybrid multimode microplate reader. The data were calculated as µM GSH using the GSH standard curve and converted to percent change from resting condition of pretraining phase.

The activity of salivary CAT, an antioxidant enzyme that can decompose hydrogen peroxide (H_2O_2) to H_2O and O_2, and acutely changes in both plasma and saliva during exercise (Berzosa et al., 2011; Souza et al., 2019), was measured using a modified version of the method reported in previous studies (Hadwan and Abed, 2016; Souza et al., 2019). A 20 µL of diluted sample was added into a 96-well ultrabiolet plate and mixed with 300 µL of 50 mM sodium and potassium phosphate buffer (pH, 7.4) containing 20 mM H_2O_2. The rate of H_2O_2 decomposition was recorded from baseline to 10 min at 2-min intervals using a hybrid multimode microplate reader at an absorbance wavelength of 240 nm. The average CAT activity/min was determined and converted to percent change from resting condition of pretraining phase. CAT standard was used as a control.

The activity of salivary SOD, an antioxidant enzyme that can decompose the free radical superoxide anion to H_2O_2 and O_2 and also acutely changes in both plasma and saliva during exercise (Souza et al., 2019; Younus, 2018) was measured by the inhibition of pyrogallol autoxidation using a method modified from previous studies (Alam et al., 2013; Souza et al., 2019). A 10 µL of salivary sample was added into a 96-well clear plate. Then 90 µL of freshly prepared 2 mM pyrogallol dissolved in Tris-HCl (pH, 8.2) containing 30 mM EDTA and 80 U/mL CAT was immedi-

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ately added into each well and then read at an absorbance wavelength of 420 nm from baseline to 10 min at 2-min intervals using a hybrid multimode microplate reader. The average increase in absorbance/minute was calculated. The SOD activity was determined as the percent change in absorbance/minute relative to resting condition of pretraining phase. For example, the increase in the absorbance/minute compared with the resting condition of pretraining phase was defined as decreased SOD activity.

Statistical analysis

The data's normality was assessed using the Shapiro–Wilks normality test. For parametric data comparisons, the data were presented as mean ± standard error of the mean. Subsequently, paired or unpaired *t*-tests, or one-way repeated-measures analysis of variance with Geisser–Greenhouse correction followed by Tukey multiple comparisons, were used to analyze the data. For nonparametric data comparisons, the data were presented as median (interquartile range). The Wilcoxon signed-rank test or Mann–Whitney *U*-tests were used to compare within each group or between groups, respectively. Moreover, the Friedman test followed by Dunn multiple comparisons test were used for within-group comparisons. Statistical analysis was conducted using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA). Specific details can be found in the table/figure legends. Statistical significance was determined by a $P < 0.05$.

Table 1. Baseline characteristics of participants

Characteristic	BW group (n = 12)	BWM group (n = 12)
Age (yr)	63.50 ± 0.79	65.83 ± 1.06
Height (cm)	156.10 ± 1.15	152.80 ± 1.99
Weight (kg)	53.70 ± 1.70	53.59 ± 1.78
Underlying diseases/conditions		
Hypertension	4	4
Type 2 diabetes mellitus	1	1
Dyslipidemia	5	4

Values are presented as mean ± standard error of the mean or number.
BW, brisk walking; BWM, brisk walking with music.
An unpaired *t*-test with Welch correction was used to compare age, height, and weight between groups.

RESULTS

Baseline characteristics of participants

The baseline participant characteristics indicated that age, height, and weight did not significantly differ between the BW and BWM groups. Additionally, the prevalence of underlying diseases/conditions, including hypertension, type 2 diabetes mellitus, and dyslipidemia, was similar in both groups, as shown in Table 1.

Changes in the BMI and body composition

The baseline BMI and body composition did not exhibit significant differences between the groups. Following an 8-week brisk walking training, no statistically significant changes were observed in BMI and body composition for the BW group. However, the 8-week BWM intervention demonstrated a reduction in percent fat mass, accompanied by an increase in percent fat-free mass ($P < 0.05$), as shown in Table 2.

Changes in the standing balance

The standing balance of participants was measured by performing a comfortable stance or a narrow stance with eyes open. The narrow stance significantly increased the SVI compared to the comfortable stance in both the BW and BWM groups ($P < 0.05$). Brisk walking training, with and without music, for 8 weeks could maintain but did not improve standing balance in this study, as shown in Table 3.

Changes in the HR and blood pressure

The baseline HR data of the pretraining phase for the BW and

Table 2. BMI and body composition of participants

Variable	BW group (n = 9)		BWM group (n = 9)	
	Pretraining	Posttraining	Pretraining	Posttraining
BMI (kg/m^2)	22.01 ± 0.63	22.21 ± 0.74	22.27 ± 0.74	22.58 ± 0.75
Fat mass (% body weight)	39.00 (31.50–40.90)	38.10 (31.70–42.25)	40.10 (36.95–41.15)	38.50 (34.40–40.40)*
Fat-free mass (% body weight)	61.00 (59.10–68.50)	61.90 (57.75–68.30)	59.90 (58.85–63.05)	61.50 (59.60–65.60)*
Skeletal muscle mass (kg)	13.69 ± 0.69	14.01 ± 0.74	12.62 ± 0.89	13.09 ± 0.86
Total body water (% body weight)	45.70 (44.10–50.85)	46.80 (43.65–50.60)	45.40 (44.40–47.00)	45.10 (44.65–48.85)

Values are presented as mean ± standard error of the mean or median (interquartile range).
BW, brisk walking; BWM, brisk walking with music; BMI, body mass index.
*$P < 0.05$ compared with pretraining phase within each group using the Wilcoxon signed-rank test.

Table 3. Standing balance of participants in pre- and post-8 weeks of brisk walking training

Variable	BW group (n=9)		BWM group (n=9)	
	Pretraining	Posttraining	Pretraining	Posttraining
Eyes open, comfortable stance				
Sway velocity index	7.87 (7.39–9.40)	7.20 (5.32–9.62)	7.23 (6.39–8.99)	7.60 (5.35–8.46)
Eyes open, narrow stance				
Sway velocity index	11.69 (10.84–12.65)*	10.97 (10.82–12.20)*	11.12 (10.29–12.10)*	11.50 (9.20–11.79)*

Values are presented as median (interquartile range).
BW, brisk walking; BWM, brisk walking with music.
*$P<0.05$ compared with eyes open, comfortable stance condition within each group using the Wilcoxon signed-rank test.

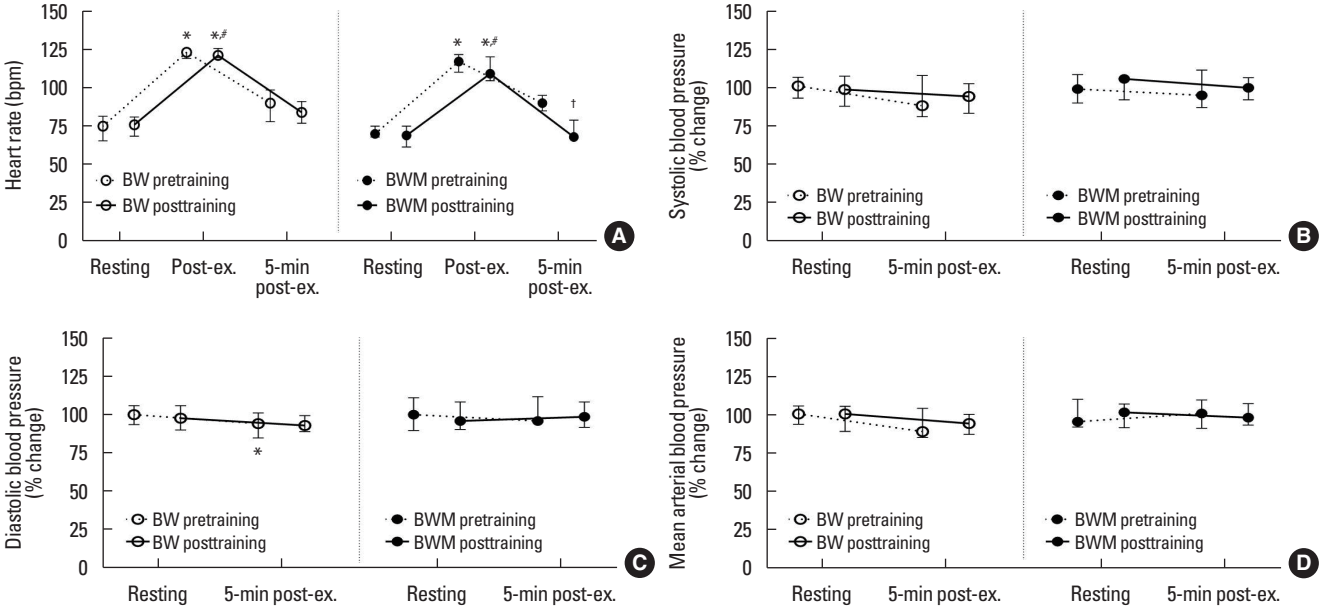

Fig. 3. Changes in heart rate and blood pressure in response to treadmill exercise testing in BW and BWM groups. (A) Heart rate. (B) Systolic blood pressure. (C) Diastolic blood pressure. (D) Mean arterial blood pressure. Data are expressed as median (interquartile range), n=9 per group. The Friedman test followed by Dunn multiple comparisons was used for statistical analysis of the data. BW, brisk walking; BWM, brisk walking with music; Post-ex., immediately postexercise; 5-min Post-ex., postexercise after 5 min cool-down. *$P<0.05$ compared with the resting condition of pretraining phase. #$P<0.05$ compared with the resting condition of posttraining phase. †$P<0.05$ compared with the immediately postexercise of posttraining phase.

BWM groups were not different, as shown in Fig. 3A. Immediately after treadmill exercise testing, participants' HRs significantly increased from resting conditions in both the BW and BWM groups ($P<0.05$) and recovered in the 5-min postexercise condition. After 8 weeks of brisk walking training, a faster HR recovery was observed in the BWM group compared to the BW group after treadmill exercise testing. The SBP, DBP, and MAP of the pretraining and posttraining phases were almost not different within groups and between groups, as shown in Fig. 3B-D. Although the DBP response to treadmill exercise testing (resting vs. 5-min postexercise) significantly decreased in the pretraining session in BW group.

Changes in HR variability

The baseline HRV parameters were not different between groups in the pretraining phase, as shown in Table 4. The average R-R interval data (mean RR), standard deviation of the RR interval, and root mean square of successive RR interval differences were significantly decreased from the resting condition during treadmill exercise testing ($P<0.05$). These parameters recovered in the 5-min postexercise testing in both groups. The percentage of adjacent RR intervals differing by more than 50 msec (pNN50) significantly decreased from the resting condition in the posttraining phase of BWM group ($P<0.05$). The ellipse width in the Poincaré plot (SD1) and the SD2 ellipse length in the Poincaré plot

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Table 4. The HRV parameters in response to treadmill exercise testing in pre- and post-8 weeks of brisk walking training

Variable	BW group (n = 9)		BWM group (n = 9)	
	Pretraining	Posttraining	Pretraining	Posttraining
Time domain				
Mean RR (msec)				
Resting	838.8 (692.7–889.8)	783.2 (732.8–920.6)	795.1 (766.9–888.7)	884.4 (847.4–994.1)
During exercise	533.3 (513.3–545.1)*	530.9 (525.8–533.9)*	539.8 (529.9–575.9)*	586.8 (555.4–637.1)*
5-min postexercise	699.9 (632.5–743.1)	705.7 (654.2–739.1)	707.9 (697.7–812.6)	817.5 (725.1–897.9)
SDNN (msec)				
Resting	29.46 (19.67–31.37)	30.31 (18.98–35.51)	18.79 (14.17–30.48)	21.03 (19.74–30.21)
During exercise	8.83 (6.40–9.20)*	6.92 (6.43–9.66)*	7.85 (5.74–10.52)*	8.11 (6.92–11.74)*
5-min postexercise	18.41 (12.55–24.13)	20.29 (14.87–26.17)	17.32 (11.64–32.51)	17.90 (15.36–26.23)
RMSSD (msec)				
Resting	18.65 (13.63–27.31)	21.41 (15.02–29.29)	16.29 (11.09–24.84)	26.14 (13.82–37.36)
During exercise	7.44 (5.53–8.30)*	6.83 (5.56–9.525)*	5.81 (4.80–9.39)*	7.63 (5.90–11.83)*
5-min postexercise	18.65 (13.63–27.31)	21.41 (15.02–29.29)	16.29 (11.09–24.84)	26.14 (13.82–37.36)
pNN50 (%)				
Resting	0.66 (0.00–6.57)	1.05 (0.15–8.60)	0.53 (0.11–4.24)	2.96 (0.59–15.77)
During exercise	0.04 (0.02–0.18)	0.00 (0.00–1.11)	0.04 (0.00–0.05)	0.12 (0.00–0.27)*
5-min postexercise	0.00 (0.00–1.11)	0.00 (0.00–0.95)	0.24 (0.00–5.70)	0.30 (0.12–3.03)
Frequency domain				
LF (n.u.)				
Resting	74.25 (59.46–83.45)	64.82 (45.92–82.75)	75.67 (61.38–78.77)	55.06 (33.86–72.52)
During exercise	68.75 (53.84–73.46)	70.67 (64.40–77.95)	63.99 (62.49–71.28)	72.39 (58.58–75.08)
5-min postexercise	81.14 (74.43–87.68)	81.00 (68.80–85.26)	78.48 (66.41–86.40)	62.15 (55.70–79.35)
HF (n.u.)				
Resting	25.73 (16.54–40.48)	35.14 (17.16–54.08)	24.26 (21.20–38.56)	44.83 (27.40–66.10)
During exercise	31.20 (26.40–46.08)	29.09 (21.97–35.45)	35.97 (28.68–37.45)	27.55 (24.84–41.32)
5-min postexercise	18.85 (12.30–25.57)	18.99 (14.74–31.17)	21.51 (13.58–33.57)	37.75 (20.62–44.21)
LF/HF				
Resting	2.89 (1.51–5.07)	1.84 (0.85–4.83)	3.12 (1.60–3.72)	1.23 (0.53–2.65)
During exercise	2.20 (1.26–2.79)	2.43 (1.82–3.63)	1.78 (1.67–2.51)	2.63 (1.58–3.03)
5-min postexercise	4.30 (3.03–7.16)	4.27 (2.26–5.80)	3.65 (1.98–6.93)	1.65 (1.28–4.11)
Nonlinear HRV				
SD1 (msec)				
Resting	13.21 (9.65–19.34)	15.16 (10.64–20.74)	11.53 (7.85–17.59)	18.51 (9.79–26.46)
During exercise	5.26 (3.92–5.87)*	4.83 (3.93–6.74)*	4.11 (3.39–6.64)*	5.39 (4.18–8.37)*
5-min postexercise	7.28 (5.05–13.84)	8.37 (5.50–13.10)	8.74 (5.83–20.20)	12.60 (8.74–15.69)
SD2 (msec)				
Resting	34.26 (25.69–41.91)	40.15 (24.67–45.77)	23.94 (17.53–38.09)	25.71 (23.07–33.84)
During exercise	10.84 (7.74–11.82)*	8.88 (7.86–11.56)*	10.31 (6.36–13.28)*	10.34 (7.52–13.95)*
5-min postexercise	21.99 (17.01–32.29)	26.89 (19.98–34.87)	23.10 (14.35–41.30)#	24.54 (18.85–30.63)
SD1/SD2				
Resting	0.37 (0.31–0.46)	0.42 (0.36–0.48)	0.44 (0.36–0.63)	0.59 (0.48–0.91)
During exercise	0.49 (0.46–0.55)	0.53 (0.44–0.63)	0.52 (0.37–0.65)	0.50 (0.48–0.56)
5-min postexercise	0.33 (0.26–0.41)	0.32 (0.27–0.41)#	0.39 (0.25–0.49)	0.52 (0.42–0.73)

Values are presented as median (interquartile range).
SDNN, standard deviation of the NN (RR) interval; RMSSD, root mean square of successive RR interval differences; pNN50, percentage of adjacent RR intervals differing by more than 50 msec; LF (n.u.), low-frequency domain in the normalized unit; HF (n.u.), high-frequency domain in the normalized unit; SD1, ellipse width in Poincaré plot; SD2, ellipse length in Poincaré plot; HRV, heart rate variability.
The Friedman test followed by Dunn multiple comparisons was used for statistical analysis.
*$P<0.05$ compared with the resting condition. #$P<0.05$ compared with the during exercise condition.

(SD2) of nonlinear HRV in both groups, reflecting sympathovagal activity (Roy and Ghatak, 2013), were significantly decreased during exercise compared with the resting condition ($P < 0.05$). The 8 weeks of brisk walking training with or without music did not change SD1 and SD2 responses. Additionally, the SD1/SD2 ratio significantly decreased in the postexercise period when compared with during exercise in the BW group after training, reflecting sympathetic dominance ($P < 0.05$).

Changes in the salivary flow rate and salivary pH

The salivary flow rate and salivary pH in both BW and BWM groups did not significantly change in pretraining and posttraining phases, as shown in Table 5.

Changes in the salivary total protein concentration and salivary antioxidant status

The salivary total protein concentration and salivary antioxidant status including salivary TAC, salivary GSH concentration, salivary CAT activity, and salivary SOD activity in both BW and BWM groups did not significantly change in pretraining and posttraining phases, as shown in Table 6.

Table 5. Salivary flow rate and salivary pH responses to treadmill exercise testing

Variable	BW group (n = 9)		BWM group (n = 9)	
	Pretraining	Posttraining	Pretraining	Posttraining
Salivary flow rate (mL/min)				
Resting	0.27 (0.23–0.37)	0.36 (0.19–0.51)	0.26 (0.16–0.32)	0.33 (0.19–0.43)
5-min postexercise	0.32 (0.23–0.36)	0.39 (0.26–0.44)	0.21 (0.20–0.26)	0.22 (0.18–0.47)
Salivary pH				
Resting	6.84 ± 0.11	6.91 ± 0.11	6.97 ± 0.12	7.00 ± 0.14
5-min postexercise	6.88 ± 0.10	6.92 ± 0.08	6.89 ± 0.11	7.05 ± 0.15

Values are presented as median (interquartile range) or mean ± standard error of the mean.
BW, brisk walking; BWM, brisk walking with music.
The Friedman test followed by Dunn multiple comparisons or one-way repeated-measures analysis of variance with Geisser–Greenhouse correction followed by Tukey multiple comparisons was used for statistical analysis.

Table 6. Salivary total protein concentration and antioxidants system responses to treadmill exercise testing

Variable	BW group (n = 9)		BWM group (n = 9)	
	Pretraining	Posttraining	Pretraining	Posttraining
Salivary total protein concentration (mg/mL)				
Resting	1.03 (0.74–1.26)	1.09 (0.81–1.38)	1.23 (0.82–1.68)	1.23 (0.75–1.50)
5-min postexercise	1.32 (1.04–1.70)	1.01 (0.91–1.51)	1.36 (1.08–1.52)	1.42 (0.72–1.66)
Salivary TAC (Trolox equivalent, μM)				
Resting	238.10 (221.80–300.20)	207.10 (131.50–260.20)	257.10 (180.20–345.60)	214.30 (170.30–300.10)
5-min postexercise	227.6 (166.40–270.40)	211.8 (195.00–235.20)	216.0 (158.50–362.60)	235.7 (170.40–297.00)
Salivary GSH concentration (% change)				
Resting	100.00 ± 19.25	122.10 ± 20.74	100.00 ± 21.92	111.10 ± 14.91
Postexercise testing	143.30 ± 26.42	119.40 ± 24.38	110.80 ± 21.01	124.00 ± 25.65
Salivary CAT activity (% change)				
Resting	72.18 (36.99–157.00)	91.08 (53.14–131.10)	91.27 (67.38–141.40)	123.80 (90.78–148.90)
5-min postexercise	85.07 (42.28–220.40)	72.18 (42.80–147.90)	115.60 (55.67–144.20)	172.90 (66.74–201.30)
Pyrogallol autooxidation (% change)				
Resting	100.00 (96.93–103.10)	97.80 (88.12–106.80)	100.00 (96.87–103.10)	102.10 (90.97–109.80)
5-min postexercise	98.90 (96.26–100.60)	101.40 (96.65–110.10)	99.35 (96.73–104.00)	100.00 (90.77–111.50)

Values are presented as median (interquartile range) or mean ± standard error of the mean.
BW, brisk walking; BWM, brisk walking with music; TAC, total antioxidant capacity; GSH, glutathione; CAT, catalase.
The Friedman test followed by Dunn multiple comparisons and one-way repeated-measures analysis of variance with Geisser–Greenhouse correction followed by Tukey multiple comparisons were used for statistical analysis. The percent change of pyrogallol autooxidation is inversely related to superoxide dismutase activity.

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DISCUSSION

Brisk walking training has been shown to have beneficial effects on health-related outcomes in the elderly, and this depends on a suitable FITT (frequency, intensity, time, type) protocol (Bai et al., 2021). Moreover, music-based interventions in physical activity can reduce the perception of exertion, increase exercise adherence, and provide benefits on health-related outcomes (Alter et al., 2015).

In our present study, brisk walking for 8 weeks can maintain but not improve body composition in older women. This may be due to various factors, including exercise duration, exercise volume, and intensity (Bai et al., 2021). Moreover, calorie intake and nutrition should be monitored. However, brisk walking with music has positive effects on fat reduction and increases fat-free mass in our study. The possible explanation may be due to fast music tempo that is synchronized with walking, which changes metabolic responses by encouraging walking faster or with a longer step length and delaying the perception of exertion. Similarly, a previous study showed that 12 weeks of walking synchronized with fast music tempo varied between 120–128 bpm, which is nearly within the music beats range of 109–132 bpm in our study, can reduce fat ratio in pre-older women (Wang et al., 2022). Moreover, the hypothalamic-pituitary-adrenal axis, particularly cortisol, has been shown to be involved in metabolic changes in response to exercise with music through its proposed mechanism of enhancing glucose and free fatty acid utilization (Ballmann et al., 2021).

Previously, brisk walking has been demonstrated to effectively prevent falls among elderly individuals living in the community (Okubo et al., 2016). The standing balance in older adults is also maintained but does not improve after 8 weeks of brisk walking training. The improvement of balance in the elderly following brisk walking training was limited by the duration of training at 8 weeks, and only static balance parameters were assessed in this study. An optimal exercise program and another balance assessment should be conducted (Paillard et al., 2004). For instance, increasing the exercise duration to at least 12 weeks and testing dynamic balance in this population.

Brisk walking has potential benefits on cardiovascular health and reduces the risks of cardiovascular diseases (Bai et al., 2021). Although our present study illustrates that 8 weeks of moderate-intensity brisk walking does not change resting HR and blood pressure, the recovery HR after treadmill exercise testing is faster in participants who previously performed brisk walking with music. It is proposed that previous experiences with music tempo during brisk walking may modulate psychophysiological adaptations, including the perception of discomfort, and cardiac autonomic regulation (Ballmann, 2021). In our study, the BWM group has potentially suppressed parasympathetic activity, as reflected by the pNN50 (%) in the HRV time domain, and subsequently reactivated after treadmill exercise testing in the posttraining phase. Meanwhile, the BW group seems to sustain sympathetic activity, as reflected by a low SD1/SD2 ratio, after treadmill exercise testing in the posttraining phase. Some inconsistent results regarding sympathovagal balance were found in exercise with or without music (Jia et al., 2016). We propose that fast music tempo during training period can modulate sympathetic tone by increasing catecholamines in systemic circulation (Yamamoto et al., 2003) and subsequently modifying cardiac autonomic function during exercise and postexercise periods. The overall sympathovagal adaptation to brisk walking training in older women in our study needs further investigation. For instance, a longer training duration of 12 weeks was used to compare cardiac autonomic regulation in Tai Chi, brisk walking, and a sedentary control group, as reported previously in older women (Audette et al., 2006).

Measurement of salivary biomarkers is a noninvasive technique that has been utilized for detecting biochemical changes related to exercise, including inflammatory biomarkers, oxidative stress, and novel biomarkers linked to diseases (Alves et al., 2022; Pacheco et al., 2022; Souza et al., 2019). In our present study, biomarkers related to exercise and antioxidant status were not significantly altered in older women performing brisk walking training corresponding with salivary flow rate and the salivary buffering system. A previous study found that an age-tailored structured intervention exercise program for 24 weeks did not alter salivary cortisol, a stress marker, but it reduced plasma oxidant level (derived-reactive oxygen metabolites) and increased antioxidant level (biological antioxidant potential) in the elderly (Morucci et al., 2022). It is suggested that optimal exercise intervention and the selection of other potential biomarkers are needed for further investigation.

This study has some limitations. Firstly, the duration of the exercise intervention needs to be longer to provide more information on its long-term effects on physiological and biochemical adaptation. Secondly, the sample size is limited due to the coronavirus disease 2019 pandemic, and we lacked a sedentary control group. Additionally, there was a high dropout rate (25%). Therefore, increasing the number of participants is needed for further investigation.

Brisk walking is a low-cost activity for older adults living in the community. Although 8 weeks of home-based brisk walking at

moderate intensity with or without music have not improved standing balance, blood pressure control, salivary biomarkers including total protein concentration, and antioxidant status, they can maintain or prevent the decline of these parameters. Only brisk walking with music can reduce fat mass relative to increasing fat-free mass and improve recovery HR by modifying cardiac autonomic control in postexercise testing. Thus, brisk walking with preferred music can be a tool to delay the progression of cardiovascular dysfunction in older women. A longer duration of the exercise program and larger groups of participants are needed for further investigation of brisk walking with or without music on physiological and biochemical changes.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharm J 2013;21:143-152.

Alter DA, O'Sullivan M, Oh PI, Redelmeier DA, Marzolini S, Liu R, Forhan M, Silver M, Goodman JM, Bartel LR. Synchronized personalized music audio-playlists to improve adherence to physical activity among patients participating in a structured exercise program: a proof-of-principle feasibility study. Sports Med Open 2015;1:23.

Alves RCC, Ferreira RO, Frazao DR, de Souza Ne YG, Mendes PFS, Maranon-Vasquez G, Royes LFF, Fagundes NCF, Maia LC, Lima RR. The relationship between exercise and salivary oxidative stress: a systematic review. Antioxidants (Basel) 2022;11:1489.

Audette JF, Jin YS, Newcomer R, Stein L, Duncan G, Frontera WR. Tai chi versus brisk walking in elderly women. Age Ageing 2006;35:388-393.

Bai X, Soh KG, Omar Dev RD, Talib O, Xiao W, Cai H. Effect of brisk walking on health-related physical fitness balance and life satisfaction among the elderly: a systematic review. Front Public Health 2021;9:829367.

Ballmann CG. The influence of music preference on exercise responses and performance: a review. J Funct Morphol Kinesiol 2021;6:33.

Ballmann CG, McCullum MJ, Rogers RR, Marshall MR, Williams TD. Effects of preferred vs. Nonpreferred music on resistance exercise performance. J Strength Cond Res 2021;35:1650-1655.

Berzosa C, Cebrian I, Fuentes-Broto L, Gomez-Trullen E, Piedrafita E, Martinez-Ballarin E, Lopez-Pingarron L, Reiter RJ, Garcia JJ. Acute exercise increases plasma total antioxidant status and antioxidant enzyme activities in untrained men. J Biomed Biotechnol 2011;2011:540458.

Ciolac EG. Exercise training as a preventive tool for age-related disorders: a brief review. Clinics (Sao Paulo) 2013;68:710-717.

Collado-Mateo D, Lavin-Perez AM, Penacoba C, Del Coso J, Leyton-Roman M, Luque-Casado A, Gasque P, Fernandez-Del-Olmo MA, Amado-Alonso D. Key factors associated with adherence to physical exercise in patients with chronic diseases and older adults: an umbrella review. Int J Environ Res Public Health 2021;18:2023.

Engelfriet PM, Jansen EH, Picavet HS, Dolle ME. Biochemical markers of aging for longitudinal studies in humans. Epidemiol Rev 2013;35:132-151.

Gronek J, Boraczynski M, Gronek P, Wielinski D, Tarnas J, Marszalek S, Tang YY. Exercise in aging: be balanced. Aging Dis 2021;12:1140-1149.

Guo J, Huang X, Dou L, Yan M, Shen T, Tang W, Li J. Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. Signal Transduct Target Ther 2022;7:391.

Hadwan MH, Abed HN. Data supporting the spectrophotometric method for the estimation of catalase activity. Data Brief 2016;6:194-199.

Hagg S, Jylhava J. Sex differences in biological aging with a focus on human studies. Elife 2021;10:e63425.

Huang CY, Chang ET, Lai HL. Comparing the effects of music and exercise with music for older adults with insomnia. Appl Nurs Res 2016;32:104-110.

Jia T, Ogawa Y, Miura M, Ito O, Kohzuki M. Music attenuated a decrease in parasympathetic nervous system activity after exercise. PLoS One 2016;11:e0148648.

Jinakote M, Yoysungnoen B, Vaddhanaphuti CS, Kasiyaphat A. Combined effects of whole-body vibration and dynamic squats on cardiovascular and salivary biomarker responses in healthy adults. J Exerc Rehabil 2023;19:280-292.

Kerksick C, Willoughby D. The antioxidant role of glutathione and n-acetyl-cysteine supplements and exercise-induced oxidative stress. J Int Soc Sports Nutr 2005;2:38-44.

Mattioli AV, Selleri V, Zanini G, Nasi M, Pinti M, Stefanelli C, Fedele F, Gallina S. Physical activity and diet in older women: a narrative review. J Clin Med 2022;12:81.

Morucci G, Ryskalin L, Pratesi S, Branca JJV, Modesti A, Modesti PA, Guli-

 https://doi.org/10.12965/jer.2448154.077

sano M, Gesi M. Effects of a 24-week exercise program on functional fitness, oxidative stress, and salivary cortisol levels in elderly subjects. Medicina (Kaunas) 2022;58:1341.

Okubo Y, Osuka Y, Jung S, Rafael F, Tsujimoto T, Aiba T, Kim T, Tanaka K. Walking can be more effective than balance training in fall prevention among community-dwelling older adults. Geriatr Gerontol Int 2016;16:118-125.

Pacheco VB, Nery G, Fernandes LL, Santana TV, Jimenez M, Borges L, Hatanaka E, Braga P, Monteiro FR, Amaral JB, Alves LS, Bachi ALL, da Silva SG, Birbrair A, Heller D. Salivary proteome, inflammatory, and netosis biomarkers in older adult practitioners and nonpractitioners of physical exercise. Oxid Med Cell Longev 2022;2022:3725056.

Paillard T, Lafont C, Costes-Salon MC, Rivière D, Dupui P. Effects of brisk walking on static and dynamic balance, locomotion, body composition, and aerobic capacity in ageing healthy active men. Int J Sports Med 2004;25:539-546.

Prasertsri P, Phoemsapthawee J, Kuamsub S, Poolpol K, Boonla O. Effects of long-term regular continuous and intermittent walking on oxidative stress, metabolic profile, heart rate variability, and blood pressure in older adults with hypertension. J Environ Public Health 2022;2022:5942947.

Roy B, Ghatak S. Nonlinear methods to assess changes in heart rate variability in type 2 diabetic patients. Arq Bras Cardiol 2013;101:317-327.

Shimamura T, Sumikura Y, Yamazaki T, Tada A, Kashiwagi T, Ishikawa H, Matsui T, Sugimoto N, Akiyama H, Ukeda H. Applicability of the dpph assay for evaluating the antioxidant capacity of food additives - inter-laboratory evaluation study. Anal Sci 2014;30:717-721.

Simioni C, Zauli G, Martelli AM, Vitale M, Sacchetti G, Gonelli A, Neri LM. Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. Oncotarget 2018;9:17181-17198.

Souza AV, Giolo JS, Teixeira RR, Vilela DD, Peixoto LG, Justino AB, Caixeta DC, Puga GM, Espindola FS. Salivary and plasmatic antioxidant profile following continuous, resistance, and high-intensity interval exercise: preliminary study. Oxid Med Cell Longev 2019;2019:5425021.

Terry PC, Karageorghis CI, Curran ML, Martin OV, Parsons-Smith RL. Effects of music in exercise and sport: a meta-analytic review. Psychol Bull 2020;146:91-117.

Wang Y, Guo X, Wang H, Chen Y, Xu N, Xie M, Wong DW, Lam WK. Training and retention effects of paced and music-synchronised walking exercises on pre-older females: an interventional study. BMC Geriatr 2022;22:895.

Yamamoto T, Ohkuwa T, Itoh H, Kitoh M, Terasawa J, Tsuda T, Kitagawa S, Sato Y. Effects of pre-exercise listening to slow and fast rhythm music on supramaximal cycle performance and selected metabolic variables. Arch Physiol Biochem 2003;111:211-214.

Younus H. Therapeutic potentials of superoxide dismutase. Int J Health Sci (Qassim) 2018;12:88-93.