

Effects of combined training on functional fitness and blood pressure in older women

Efeitos do treinamento combinado na aptidão funcional e pressão arterial em mulheres idosas

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ABSTRACT

Objective: To evaluate the effects of combined training (CT) on functional fitness and blood pressure in older women. **Methods:** Sixty-seven elderly women were randomized into two groups: CT (n = 35; 67.03 ± 4.41 years) and control (CG; n = 32; 67.69 ± 6.53 years). The intervention lasted 12 weeks, with three weekly sessions of 50 minutes. The protocol included warm-up with walking and squats, 32 minutes of resistance exercises on machines, 12 minutes of walking/running and stretching. Functional fitness was assessed by the following tests: shirt-on-shirt-off test (SFT); 10-meter walk (C10m); time-up-and-go (TUG); standing-up from the floor (SF); sitting-up from a chair – 5rep (SFC5r); gallon-jug shelf-transfer (GJST); and 6-minute walk test (C6min). Blood pressure (BP) was measured with a sphygmomanometer before and after CT. **Results:** At the end of the intervention, CT showed statistically significant adaptations in all physical abilities and BP in relation to the initial values. When compared to the CG, CT promoted significant improvements in the C10m ($\Delta\%$: 7.07; $p \leq 0.001$); TUG ($\Delta\%$: 9.62; $p \leq 0.001$); C6min ($\Delta\%$: 6.49; $p = 0.002$); SF ($\Delta\%$: 19.6; $p \leq 0.001$); SFC5r ($\Delta\%$: 10.3; $p \leq 0.001$); SFT ($\Delta\%$: 11.9; $p \leq 0.001$); GJST ($\Delta\%$: 9.6; $p \leq 0.001$) and systolic blood pressure ($\Delta\%$: 6.0; $p \leq 0.001$). **Conclusion:** This protocol has been shown to be effective in reducing systolic blood pressure and improving functional fitness in independent older women.

Keywords: cardiovascular health; aging; resistance training; daily activities.

RESUMO

Objetivo: Avaliar os efeitos do treinamento combinado (TC) na aptidão funcional e pressão arterial de idosas. **Métodos:** Sessenta e sete mulheres idosas foram randomizadas em dois grupos: TC (n = 35; 67,03 ± 4,41 anos) e controle (GC; n = 32; 67,69 ± 6,53 anos). A intervenção teve duração de 12 semanas, com três sessões semanais de 50 minutos. O protocolo envolveu aquecimento com caminhada e agachamentos, 32 minutos de exercícios resistidos em máquinas, 12 minutos de caminhada/corrida e alongamentos. A aptidão funcional foi avaliada pelos testes: teste vestir e tirar a camisa (VTC); caminhada de 10 metros (C10m); time up and go (TUG); levantar do solo (LS); sentar e levantar da cadeira (SL5r); transferência de galões (GJST) e teste de caminhada em 6 min (C6min). A pressão arterial (PA) foi medida com esfigmomanômetro antes e após TC. **Resultados:** Ao final da intervenção, o TC apresentou adaptações estatisticamente significativas em todas as habilidades físicas e na PA com relação aos valores iniciais. Quando comparado ao GC, o TC promoveu melhoras significativas nos testes de C10m ($\Delta\%$: 7,07; $p \leq 0,001$); TUG ($\Delta\%$: 9,62; $p \leq 0,001$); C6min ($\Delta\%$: 6,49; $p = 0,002$); LS ($\Delta\%$: 19,6; $p \leq 0,001$); SL5r ($\Delta\%$: 10,3; $p \leq 0,001$); VTC ($\Delta\%$: 11,9; $p \leq 0,001$); GJST ($\Delta\%$: 9,6; $p \leq 0,001$) e na Pressão arterial sistólica ($\Delta\%$: 6,0; $p \leq 0,001$). **Conclusão:** O presente protocolo demonstra-se eficaz na atenuação da pressão arterial sistólica e na melhora da aptidão funcional em idosas independentes.

Palavras-chave: saúde cardiovascular; envelhecimento; treinamento resistido; atividades diárias.

Introduction

A progressive decline in cardiorespiratory and musculoskeletal system function accompanies the aging process, leading to a reduction in functional fitness and an increased prevalence of chronic diseases, including systemic arterial hypertension (SAH) [1], which affects over 60% of older adults [2].

From this perspective, the literature indicates an association between chronic diseases and the inability to perform daily activities in older adults [3]. Furthermore, regular physical exercise can preserve or even improve functional fitness and cardiovascular health, thereby delaying the onset of aging-related limitations [4].

There is a consistent body of evidence regarding the hypotensive effect of acute [5,6] and chronic interventions involving aerobic training (AT) and resistance training (RT) individually [7,8]. A recent meta-analysis shows that AT reduces systolic blood pressure (SBP) by 7.79 mmHg and diastolic blood pressure (DBP) by 5.97 mmHg, while RT reduces SBP by 5.62 mmHg and DBP by 1.87 mmHg, respectively [9].

In this context, combined training (CT) is a physical training method that can mitigate functional declines associated with senescence. This approach integrates neuromuscular and cardiorespiratory exercises within the same session [10], and its effects on body composition [11] and physical fitness in older adults have already been proven [12]. However, its potential as an antihypertensive therapy remains unclear. Therefore, the present study aimed to analyze the effects of CT on functional fitness and blood pressure in independent older women. We hypothesized that integrated exercise protocols are efficient in promoting multisystem adaptations in older adults.

Methods

The current study comprised a 16-week randomized controlled trial, with 12 weeks dedicated to the application of the intervention (TC), two weeks allocated for data collection, and two weeks for familiarization. As such, the present proposal adheres to the CONSORT recommendations (<http://www.consort-statement.org>). Additionally, some hypotheses related to this topic were published prior to the initiation of this project [13,14].

Furthermore, to ensure the rights (autonomy, non-maleficence, beneficence, justice, and equity) of all research participants and to fulfill the bioethical responsibilities advocated by the World Medical Association through the Declaration of Helsinki (1964) and by the National Health Council in Resolution No. 466/2012, the study was submitted to the Research Ethics Committee involving Human Beings (CEP/CONEP). It underwent evaluation and adaptation to current regulations and was subsequently approved by the Federal University of Sergipe's Ethics Committee (Opinion No.: 3.225.938). The study was registered with the Brazilian Registry of Clinical Trials (ReBEC) under protocol RBR-2d56bt.

Sampling procedures

To apply the protocol and for subsequent analysis, older women who met the following intervention criteria were selected: (1) Age between 60 and 80 years; (2) Not engaged in physical training programs within three months prior to the study's commencement; (3) Ability to move independently without support; (4) Cognitive status score ≥ 14 on the Mini-Mental State Examination [15]; (5) Possession of medical clearance. Participants with the following characteristics were not included in the study: (1) Stage 2 hypertension (systolic ≥ 160 mmHg and diastolic ≥ 100 mmHg); (2) Any degenerative joint disease or joint implants; (3) Cardiovascular and/or pulmonary disease that precluded high-intensity exercise; (4) Neurological deterioration. Furthermore, participants who missed any intervention stage or who completed less than 85% of the training sessions were excluded from the analyses.

One hundred and sixteen independent older women expressed interest in participating in the study. Of these, 36 were excluded for not meeting the inclusion criteria, and 13 did not complete all intervention stages (Figure 1). Consequently, 67 participants were allocated by stratified block randomization, with participants equally distributed based on lower limb strength into two distinct groups: (CT: $n = 35$; 67.03 ± 4.41 years; 27.58 ± 3.98 kg/m²) and (CG: $n = 32$; 67.69 ± 6.53 years; 28.58 ± 4.54 kg/m²).

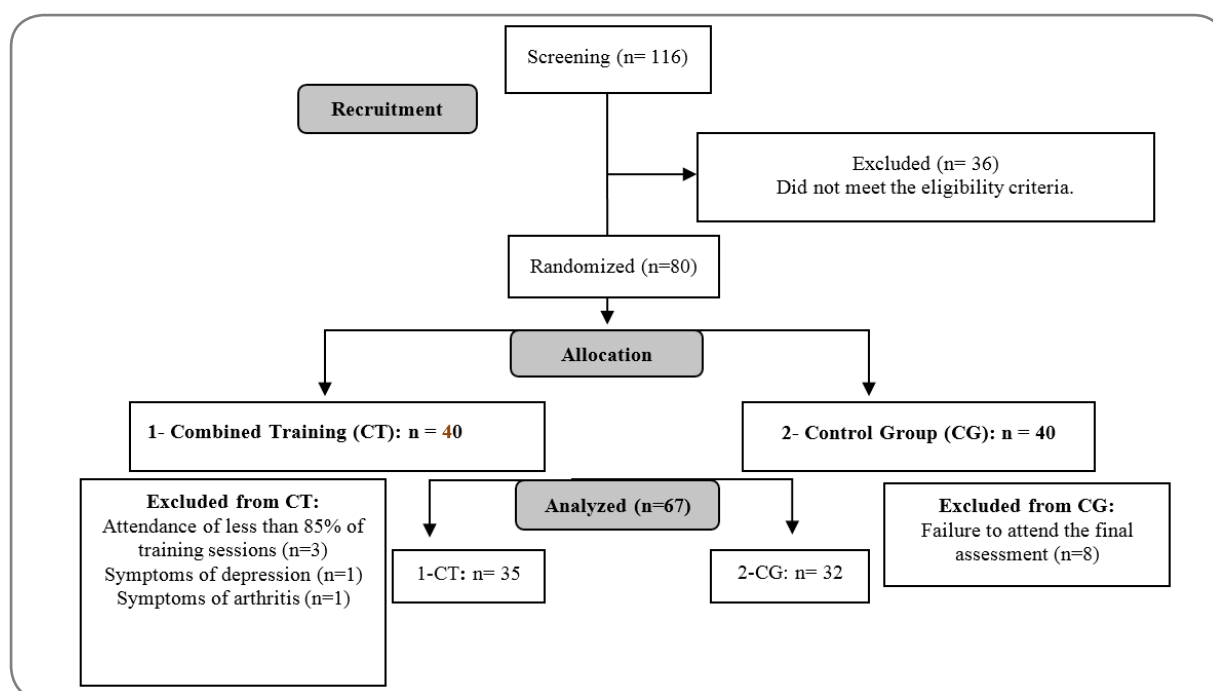


Figure 1 - Schematic representation of the study screening, allocation and intervention processes

Physical training program

All sessions were conducted at a consistent time for all volunteers to ensure homogeneity and minimize biases that could influence blood pressure analysis. There were 36 training sessions in total, comprising 3 sessions per week, each lasting 50 minutes, with a minimum recovery period of 48 hours between sessions.

Following initial assessments, participants in the CT group underwent two weeks of familiarization, during which they performed exercises at 50% of the intensity planned for the first session. After this period, they engaged in supervised, individualized, and progressive training. Progression was achieved through daily quantification and multiplication of the lifted weight and volume (Volume load = sets x repetitions x load) for the eight exercises in the neuromuscular block [16].

Combined training

The protocol was structured within a single training session, comprising four distinct exercise blocks. Block (1) consisted of a general and specific warm-up. Block (2) involved circuit-based resistance training using machines, targeting major muscle groups. Block (3) focused on endurance training through interval running, and block (4) incorporated submaximal stretching for the main muscle groups [14]. Box I provides a detailed description of the training protocol.

Box 1 – Detailed description of the combined training protocol

Blocks	Exercises	Training phase (1st to the 36th session)
Blocks I	2 minutes of walking + 15 squats at maximal concentric velocity	Total time: 3 min; 2 min walk; 1 set – 15 squats; RPE: 4-5
Blocks II	Free Squat, Articulated Chest Press, Bilateral 45° Leg Press, Pronated Lat Pulldown, Bilateral Leg Curl, Hip Abduction (Machine), Bilateral Leg Extension, and Calf Raise.	Total time: 32 min; 8 exercises; 2 rounds; 40s work/80s rest per station (20s transition) - Density 1/2; RPE: 7-9. Total time: 12 min; 30s work/60s rest – Density 1/2; RPE: 6-7.
Blocks III	Light walk/jog	Total time: 32 min; 8 exercises; 2 rounds; 40s work/80s rest per station (20s transition) - Density 1/2; RPE: 7-9. Total time: 12 min; 30s work/60s rest – Density 1/2; RPE: 6-7.
Blocks IV	Submaximal stretching for major muscle groups	Total time: 3 min; Density 1:1; RPE 4-6.

Min = minutes; s = seconds; RPE = Rating of Perceived Exertion.

In Block II, intensity was controlled using a submaximal repetition range (8 to 12 reps). The criterion for terminating a set was a reduction in concentric movement velocity exceeding three seconds. If participants completed more repetitions than prescribed, the weight was increased by 5-10% to maintain the predetermined repetition range. Similarly, if the desired repetition range was not achieved, the weight was reduced.

The Control Group (CG) was instructed to maintain their usual activities. To help retain participants throughout the intervention, lectures on senior health and rights were offered every 15 days over the 12-week intervention period.

Data collection procedures

Functional fitness was assessed using specific tests designed to analyze performance in essential skills such as walking, rising from a chair, and dexterity. These skills are crucial for maintaining independence and overall quality of life. A detailed description of all tests follows:

1) 10-Meter Walk Test (C10m)

Maximal walking speed was assessed using the 10-meter walk test (C10m). The volunteer was instructed to walk a 12.4-meter course without any assistance, in the shortest time possible [17]. Four cones and a tape measure were used to mark a straight 12.4-meter path on the floor. The volunteer walked 1.2 meters for acceleration, 10 meters for time recording, and 1.2 meters for deceleration. Two trials were performed with a two-minute rest interval between them. The test was invalidated if the volunteer exhibited a flight phase, characterizing a run [18].

2) Timed Up and Go (TUG)

Lower-limb functional fitness, emphasizing mobility, speed, agility, and dynamic balance, was assessed using the Timed Up and Go (TUG) test. The test involved measuring the time taken to complete a 3-meter walk with a change in direction. Volunteers were instructed to sit in a chair with their back against the rest, arms at their sides, and feet parallel. Upon the verbal command “go” from the evaluator, they stood up, walked the 3-meter distance at a rapid, safe, and comfortable pace, turned around, and sat back down. Three attempts were performed, with a one-minute rest interval between each. The average of the obtained results was used for final analysis. Any attempt was invalidated if the volunteer made contact with the cone during the course. Additionally, the TUG test demonstrates good reliability with an ICC of 0.99 [19].

3) Six-Minute Walk Test (C6min)

Cardiorespiratory capacity was assessed by measuring the distance covered while walking as fast as possible for six minutes. The protocol utilized a marked 40-meter linear course with sub-markings every five meters to facilitate accurate distance measurement. Upon the evaluator’s command (“go”), the volunteer began the test, walking at a rapid and consistent pace (but not running) in a straight line to the 40-meter mark. They then rounded the mark and returned to the starting point, repeating this circuit until the six-minute timed duration of the test was completed. This test demonstrates good reliability with an ICC of 0.99 [20].

4) Standing-up from the floor (SF)

For global functionality, the SF test was used, in which the volunteer was instructed to lie in a prone position, with her arms parallel to her torso and her palms facing upwards, and her face against the ground on her preferred side. On the

evaluator's command ("now"), the volunteer stood up in her usual way and tried to stay in a balanced position, with her feet parallel and pointing forward, and without moving, in the shortest time possible. A mat was used for support, and a stopwatch was used to mark the time [21].

5) Sitting-up from a chair – 5r (SFC5r)

Lower limb functional fitness, focusing on muscle strength and power in a functional action, was assessed using the SFC5r test. The volunteer stood up from the chair and returned to the starting position five times, as quickly as possible, after the evaluator's command ("now"). Three attempts were made with an interval of 1 minute. The measure was invalidated when the participant did not touch the seat or remove her arms from her torso. It also has a good ICC reliability of 0.95 and a minimum detectable difference of 2.5s [22].

6) Shirt-on-shirt-off test (SFT)

The SFT test was used to assess upper limb coordination. The participant was asked to stand with her arms parallel to her torso, holding a T-shirt in her dominant hand. When the assessor gave the command ("now"), she had to put the shirt on completely and then take it off, removing it if necessary, and holding it again with her dominant hand, returning to the starting position in the shortest possible time. Furthermore, the test has an ICC of 0.75, showing good reliability [21].

7) Gallon-jug shelf-transfer (GJST)

The GJST was used to assess functional fitness with an emphasis on the upper limbs, in an everyday action of transferring gallons on a shelf. The volunteer was instructed to stand to the side of a bookcase (2.13 x 1.06 m with adjustable shelves) with one shelf positioned at patella height, another at shoulder height, and five gallons (3.99 kg each) positioned on the bottom shelf next to each other, where they transported the gallons from one shelf to the other in the shortest time possible. The attempt was not validated if the participant moved more than one gallon at a time or switched hands to perform the movement. In addition, this test was reliable with an ICC of 0.97 [23].

8) Determinants of blood pressure

To assess blood pressure parameters, all BP measurements were taken on the left arm, with the volunteers sitting with their feet flat on the floor, in silence for 30 minutes and in a rest room, air-conditioned to 25°C, using a properly calibrated and appropriate sphygmomanometer. A cuff selected after measuring the circumference of each participant's arm (Sanny, São Paulo, Brazil) was used and placed at the midpoint of the left arm.

The procedure was used to obtain SBP, DBP and HR. The average of each volunteer's three assessments was used in the final analysis.

Statistical procedures

Descriptive analysis, including mean, standard deviation, and percentage delta, was used to characterize all obtained variables. Sample size was calculated using G*Power software (version 3.1.9.2) based on the main functional fitness variables from results reported by Resende-Neto *et al.* [14]. Anticipating an average 10% improvement in participant performance, a power of 0.80 was considered for the analyses performed in the present study's sample size calculation.

Normality was confirmed by the Shapiro-Wilk test, and homogeneity by Levene's test. A 2x2 ANOVA with Bonferroni post-hoc analysis was conducted for all analyses. Data were tabulated and analyzed using JAMOV software, version 2.4, with a significance level set at 5% ($p \leq 0.05$). All tests were two-tailed, and the effect size (ES) was calculated according to the methodological procedures [24].

Results

The combined training (CT) protocol showed a 96% adherence rate among participants. In the CT group, two participants were lost due to medical exemptions, and three were lost due to attendance falling below 90%. Conversely, the control group (CG) experienced eight losses because participants did not complete all study stages (Figure 1). The average participation rate was 34.5 out of 36 total sessions, resulting in a sample loss of 13 individuals. Anthropometric characteristics of both groups are presented in Table I; no significant differences were observed in these values.

Table I - Characteristics of participants in the Combined Training (CT) and Control (CG) groups at baseline. Values are presented as mean, standard deviation, and percentage

Variables	CT (n = 35)	CG (n = 32)	p
Age (years)	67.03 ± 4.41	67.69 ± 6.53	0.628
Height (m)	1.53 ± 0.06	1.53 ± 0.06	0.693
Weight (kg)	64.81 ± 9.77	66.63 ± 11.65	0.489
BMI (kg/m) ²	27.58 ± 3.98	28.58 ± 4.54	0.337
Diabetes	35.9%	50%	
Hypertension	66.6%	75%	
Dyslipidemia	58.9%	50%	
Anxiety	15.3%	12.5%	

* BMI = Body mass index. % = Have the pathology.

Figure 2 shows that the total training load (Volume load) was progressive throughout the training period.

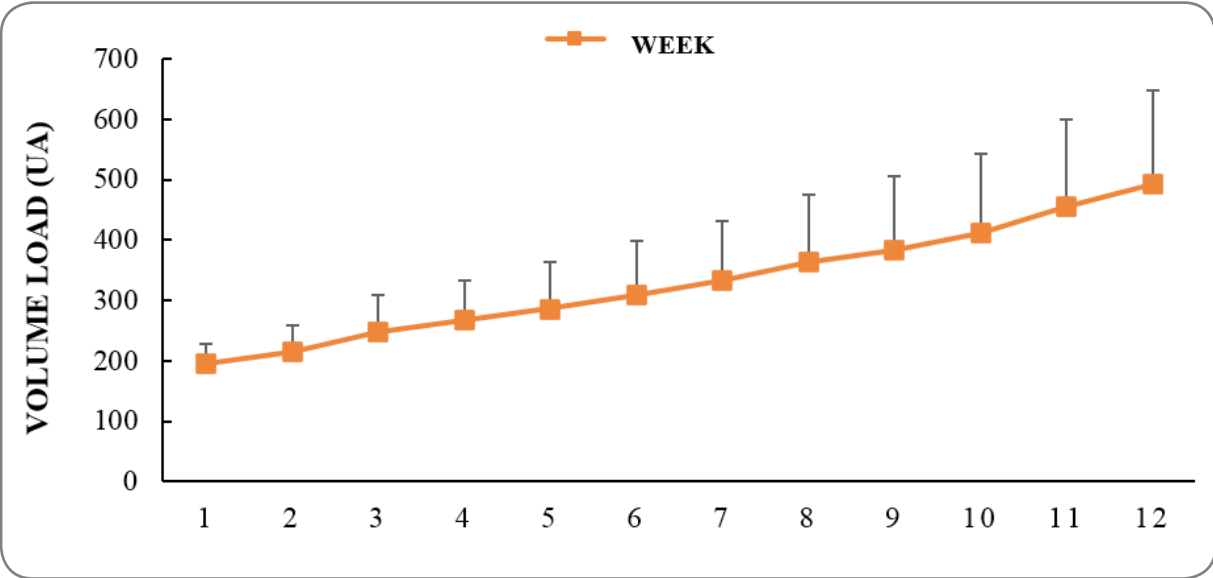


Figure 2 - Representação esquemática do comportamento do volume load ao longo de 12 semanas de treinamento combinado

At the end of the intervention, CT showed statistically significant improvements in all the variables compared to the initial values. When compared to the CG, CT promoted significant adaptations in the following variables: 1) C10m ($\Delta\%$: 7.07; $p \leq 0.001$); 2) TUG ($\Delta\%$: 9.62; $p \leq 0.001$); 3) SF ($\Delta\%$: 19.6; $p \leq 0.001$); 4) SFC5r ($\Delta\%$: 10.03; $p \leq 0.001$); 5) SFT ($\Delta\%$: 11.9; $p \leq 0.001$); 6) GJST ($\Delta\%$: 9.6; $p \leq 0.001$). The detailed results of the interventions in the tests applied are shown in Table II.

Table II - Effects of 12 weeks of combined training on functional fitness and blood pressure in independent and untrained elderly women

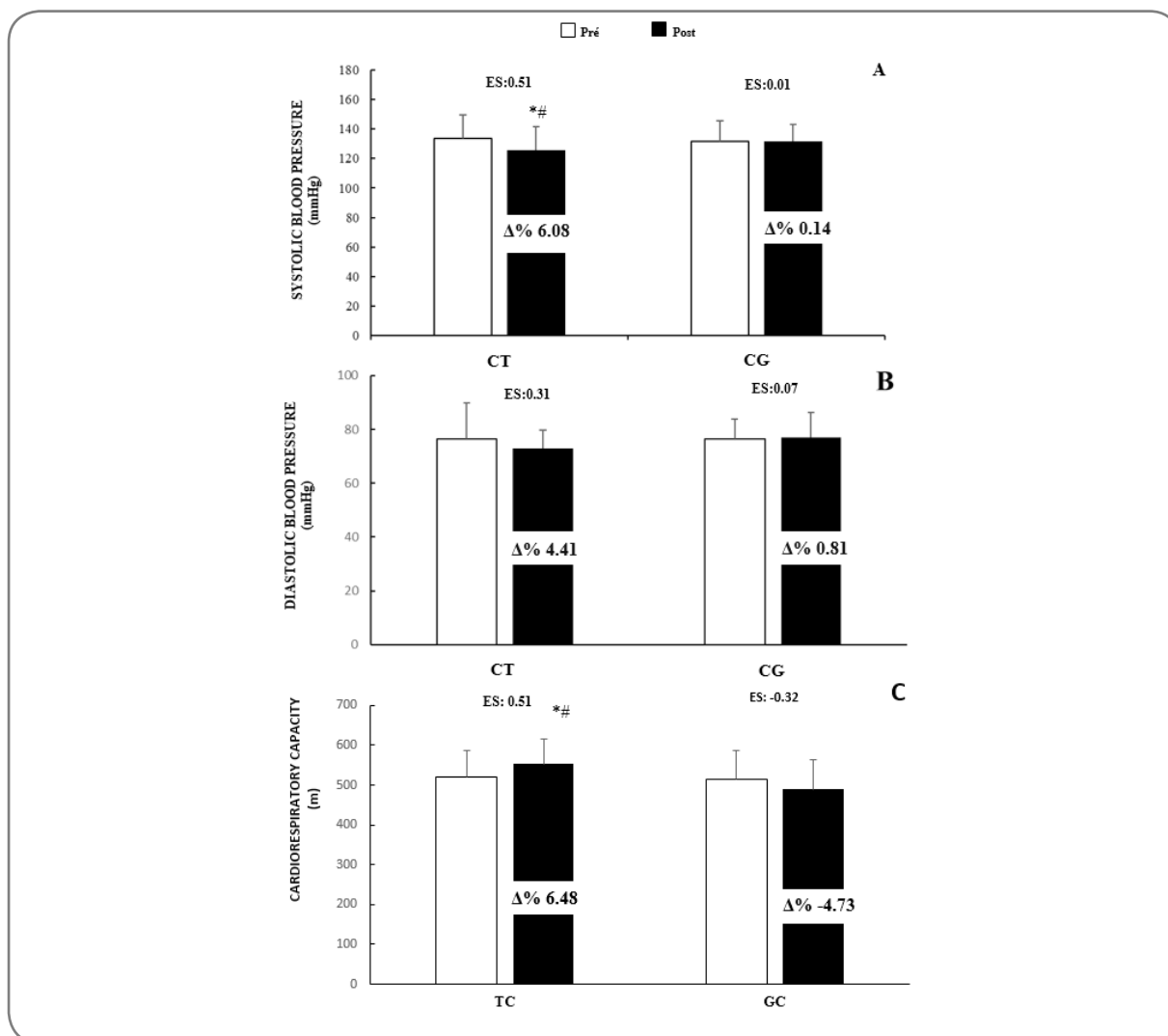
Moments	Combined	Control	p-Value Interaction Group - Time	Combined vs Control
Walking ability				
10-Meter Walk Test (s)				
Baseline (prE)	4.84 ± 0.55	5.12 ± 0.81		
POsT_12_WEEK	4.52 ± 0.57 ^{*#}	5.06 ± 0.72	0.014	0.001
Δ% - ES	7.07 – 0.57 ^M	1.17 – 0.07		
IC (95%)	(4.45-4.90)	(4.85-5.32)		
Levantando e caminhar (s)				
Baseline (prE)	5.92 ± 0.70	6.22 ± 0.84		
POsT_12_WEEK	5.35 ± 0.56 ^{*#}	6.15 ± 0.91	0.001	0.001
Δ% - ES	9.62 – 0.89 ^G	1.12 – 0.07		
IC (95%)	(5.38-5.89)	(5.91-6.45)		

Table II - Continuation

Moments	Combined	Control	p-Value Interaction Group - Time	Combined vs Control
Ability to lift				
Standing-up from the floor (s)				
Baseline (prE)	3.31 ± 1.40	3.50 ± 1.38		
POsT_12_WEEK	2.66 ± 0.90 ^{*#}	3.51 ± 1.06	0.001	0.001
Δ% - ES	19.6 - 0.55 ^M	0.28 - -0.08		
IC (95%)	(2.58-3.39)	(3.50-3.92)		
Sitting-up from a chair - 5r (s)				
Baseline (prE)	8.03 ± 1.11	7.58 ± 1.71		
POsT_12_WEEK	7.20 ± 1.21 ^{*#}	7.96 ± 1.78	0.001	0.001
Δ% - ES	10.30 - 0.71 ^G	5.01 - -0.21		
IC (95%)	(7.12-8.11)	(10.79-12.33)		
Dexterity				
Shirt-on-shirt-off test (s)				
Baseline (prE)	14.19 ± 3.09	14.99 ± 2.78		
POsT_12_WEEK	12.49 ± 2.47 ^{*#}	14.65 ± 2.81	0.001	0.001
Δ% - ES	11.9 - 0.60 ^M	2.26 - 0.12		
IC (95%)	(12.31-21.72)	(20.75-15.8)		
Gallon-jug shelf-transfer (s)				
Baseline (prE)	11.11 ± 1.42	11.21 ± 1.92		
POsT_12_WEEK	10.04 ± 1.18 ^{*#}	11.17 ± 1.62	0.001	0.001
Δ% - ES	9.6 - 0.81 ^G	0.35 - 0.02		
IC (95%)	(15.33-11.1)	(10.6-17.65)		

Values presented as mean and standard deviation (M ± SD). s = seconds; * p ≤ 0.05 vs. Baseline (pre). # p ≤ 0.05 vs. Control. Δ% = Percentage change between Baseline (pre) and Post_12. ES = Effect size (SSmall: 0.2 - 0.4; MMedium: 0.5 - 0.7; LLarge: 0.8 - 1.33; VLVery large: > 1.33). IC - Interval Confidence

About cardiorespiratory variables, HT led to statistically significant improvements compared to CG in the 6-minute walk test (C- Δ%: 6.49; p < 0.002); and in SBP (A - Δ%: 7.9; p ≤ 0.001). However, no significant changes were observed in DBP (B- Δ%: 4.41; p = 0.273) (Figure 3).



ES: Effect size. $\Delta\%$: Percentage change between Baseline (pre) and Post; m = meters; * $p \leq 0.05$ vs. Baseline (pre). # $p \leq 0.05$ vs. Control.

Figure 3 – Effects of 12 weeks of combined training on Blood Pressure and Cardiorespiratory Capacity

Discussion

The main finding of this investigation was the positive effects of CT on functional fitness and systolic blood pressure in independent older women. The combination of neuromuscular and metabolic stimuli in the same session yields significant adaptations in functionality, as previously demonstrated by our group [14,25,26]. It is essential to note that this manuscript provides a precise weekly quantification of the training load; therefore, we can infer that there was no stagnation of the effects throughout the intervention period, and this progressive stimulus partly explains the functional improvements [27].

Gait ability, analyzed using the 10m C and TUG tests, showed significant improvement. This response may be related to the increased motor coordination, dynamic balance, and muscular power provided by the main blocks of the CT [14,28]. Important findings suggest that programs integrating strength and aerobic exercises

are essential for achieving faster, more stable, and safer gait, thereby improving the quality of life for older individuals or those with physical frailty [1,29-31].

The improvement in lifting ability, observed in the SL5r and LS tests, can be explained by neuromuscular adaptations caused by the set of resistance exercises in block 2, in which a moderate to high intensity was progressively applied (Figure 2), and the participants were instructed to achieve maximum concentric speed in the repetitions. In this perspective, Souza *et al.* [32] demonstrated that exercises performed at high-speed lead to effective changes in muscle power and functionality in older women.

Multi-joint resistance exercises for the upper and lower limbs promote greater activation of motor units and improved motor control, which are essential for improving dexterity in older adults [33]. Therefore, significant improvements were observed in the GJST and VTC with the proposed CT protocol.

Regarding cardiorespiratory fitness, the CT protocol promoted significant improvements with a moderate effect, which may be due to the cardiometabolic block that combined walking with light jogging for 12 minutes over 12 weeks. This type of stimulus has the potential to cause positive changes in central and peripheral mechanisms that favor oxygen transport and utilization [34].

Furthermore, significant evidence demonstrates a parallel relationship between improved cardiorespiratory fitness and blood pressure reduction [34,35], which was also observed in the present study. In this sense, the notion that strength and aerobic exercises can generate a synergistic effect, producing different adaptations, enhancing vascular benefits, increasing endothelium-dependent dilation, and reducing arterial stiffness, is supported [35].

The combined intervention significantly reduced participants' SBP, as observed in other studies [9]. This systematization can lead to peripheral and central changes favorable to BP reduction, such as improved endothelial function with a consequent reduction in peripheral vascular resistance [6,36,37], reduced sympathetic activity, improved baroreflex sensitivity, and increased nitric oxide production, which promotes vasodilation and significantly reduces arterial stiffness [38].

The lack of significant results in DBP may be related to the fact that peripheral resistance control is more influenced by sympathetic tone and structural factors of blood vessels, which may take longer to adapt to training [39]. Thus, Moraes *et al.* [40] demonstrated a greater impact of CT on SBP compared to DBP after 12 weeks of intervention. However, it is important to note that our protocol resulted in a 3.37 mmHg reduction in DBP, and studies show that reductions above two mmHg can reduce the prevalence of coronary heart disease by between 9.2 and 18.4%, stroke by between 5.6 and 17.5%, and hypertension by 17% [41].

The study's limitations include the lack of dose control and standardization of antihypertensive medication intake. However, maintaining daily habits favors external validity and appears not to have influenced the blood pressure response to training. Therefore, we suggest that future interventions include greater control of

intervening factors such as diet and medication use, as well as comparing CT with multicomponent methods, such as functional training.

Conclusion

Combined training proves effective in improving functional fitness and attenuating systolic blood pressure in older women. Thus, this investigation demonstrates that a physical training program designed to promote health benefits for older adults should focus on the integrated enhancement of cardiorespiratory and neuromuscular fitness.

Conflicts of interest

No conflicts of interest relevant to this study were reported.

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Author's contribution

Conception and design of research: Resende Neto AG; Data acquisition: Resende Neto AG, Cruz CAB, Getirana-Mota M; Data curation: Santos LES, Resende Neto AG; Data analysis and interpretation: Santos LES, Resende Neto AG; Statistical analysis: Santos LES, Resende Neto AG; Manuscript writing: Santos LES, Resende Neto AG, Da Silva-Grigoletto ME; Critical review of the manuscript for important intellectual content: Resende Neto AG.

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