

## EFFECTIVENESS OF 24 WEEKS OF WALKING WITH RESTRICTED BLOOD FLOW ON MUSCLE ACTIVATION AND STRENGTH IN OVERWEIGHT ELDERLY WOMEN WITH OSTEOPOROSIS: A RANDOMIZED CLINICAL TRIAL

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### ABSTRACT

The present study aimed to analyze the effect of 24 weeks of AT with RFS on muscle activation and strength in overweight elderly women with osteopenia/osteoporosis. Thirty elderly women [66 (5) years] were randomly assigned to one of three interventions: CAM (moderate-intensity walking), CAM+RFS (low-intensity walking with RFS), or RFS (RFS alone). Muscle activation (EMGS) and knee flexor and extensor strength were measured, respectively, by surface electromyography and dynamometry, pre-

intervention and after 12 and 24 weeks. Knee extensor EMGs and knee flexor strength increased similarly across all interventions at 24 weeks ( $p<0.05$ ). A moderate effect size (ES, Cohen's  $d$ ) was observed in knee extensor EMGs (ES=0.65) for CAM+RFS compared to CAM. The ESs for knee flexor strength were  $-0.86$  and  $-0.69$ , respectively for RFS and CAM+RFS compared to CAM. Therefore, interventions based on the RFS method showed similar strength gains, however, elderly women can achieve long-term neuromuscular gains over 24 week

**Keywords:** aging, aerobic exercise, vascular occlusion, electromyography.

## EFICÁCIA DE 24 SEMANAS DE CAMINHADA COM RESTRIÇÃO DO FLUXO SANGUÍNEO NA ATIVAÇÃO E FORÇA MUSCULAR EM IDOSAS COM SOBREPESO E OSTEOPOROSE: UM ENSAIO CLÍNICO RANDOMIZADO

### RESUMO

O presente estudo objetivou analisar o efeito de 24 semanas de TA com RFS na ativação e força muscular de idosas com excesso de peso e osteopenia/osteoporose. Trinta mulheres idosas [66 (5) anos] foram aleatoriamente designadas para uma das três intervenções: CAM (caminhada de intensidade moderada), CAM+RFS (caminhada de baixa intensidade com RFS) ou RFS (RFS sozinho). A ativação muscular (EMGS) e a força dos flexores e extensores do joelho foram mensuradas, respectivamente, por eletromiografia de superfície e dinamometria, pré-intervenção e após 12 e 24 semanas. O EMGS dos

extensores do joelho e a força dos flexores do joelho aumentaram de forma semelhante em todas as intervenções em 24 semanas ( $p<0,05$ ). Um tamanho de efeito moderado (ES, Cohen's  $d$ ) foi observado na EMGS dos extensores do joelho (ES= 0,65) para o CAM+RFS em comparação com o CAM. Os ESs para a força dos flexores do joelho foram  $-0,86$  e  $-0,69$ , respectivamente para RFS e CAM+RFS em comparação com CAM. Portanto, as intervenções baseadas no método de RFS apresentaram ganhos de força semelhantes, no entanto, mulheres idosas podem alcançar ganhos neuromusculares de longo prazo ao longo de 24 semanas.

**Palavras-chave:** envelhecimento, exercício aeróbico, oclusão vascular, eletromiografia.

## 1. INTRODUCTION

The aging process leads to a natural and multifactorial loss of muscle strength and power with a no associated muscle or neurological diseases called dynapenia (Hackney, Brown, Stone & Tennent, 2018). Elderly presents high prevalence of dynapenia, increased functional disability and risk of falls (Cook SB, LaRoche DP, Villa MR, Barile H & Manini TM, 2017). In turn, osteoporosis causes bone fragility, with a consequent increased risk of fractures due to aged-induced deterioration of bone tissue microarchitecture (Howe et al, 2011). Moreover, previous studies have reported the risk of some non-spine fractures (e.g. proximal humerus, ankle, and upper leg fracture) in older adults with excess adipose tissue (Kelly, Gilman, Boschiero & Ilich, 2019; Scott et al, 2016). Thus, recognizing the comorbidities in elderly people (osteopenia/osteoporosis, dynapenia, and overweight/obesity) may help to guide an planned treatment as opposed to treating each single disease state (Kelly et al., 2019).

To improve the bone health of the elderly, especially with osteopenia/osteoporosis, interventions should promote stimulus to increase bone mineral density, such as mechanical stimuli (mechanostat theory) (Torres-Costoso, Lopez-Munoz, Martinez-Vizcaino, Alvarez-Bueno, Caverro-Redondo, 2020) and increased muscular glycogen metabolism (Kanazawa et al, 2009). Additionally, physical exercise can improve muscle activation and strength, decreasing episodes of falls and possible fractures in the elderly (Clark & Manini, 2012). For these reasons, traditional resistance training and moderate-vigorous intensity aerobic training (AT) have been commonly recommended to improve health and to mitigate aging-related physical disability (Haskell et al, 2007). According to (kneffel, Murlasits, Reed, Krieger, 2021), in a study carried out in Hungary, Qatar and the United States compared muscle strength and hypertrophic adaptations to resistance training programs carried out with different training frequencies in adults over 60 years of age and found that improvements in upper and lower limb strength were dependent on the number of training days and that the frequency of resistance training did not affect muscle hypertrophy. Another study carried out in Spain (i Iranzo, Balasch-Bernat, Tortosa-Chuliá, Balasch-Parisi, 2018) with a group of 37 elderly people with sarcopenia, compared the effects of two resistance training programs on peripheral and respiratory muscles on muscle mass and strength and physical performance. And they concluded that the training groups showed improvements in maximum static inspiratory and expiratory pressure, knee extension and arm flexion and there was no significant change in gait speed in any of the groups studied. However, countless older people have limitations to perform a traditional exercise program due to comorbidities, high mechanical stress to bones and joints (Centner, Wiegel, Gollhofer & Konig, 2019), high loads, and available time by session. In that perspective, blood flow restriction (BFR) has been investigated as an alternative method combined with resistance training or AT to promote different health benefits for older people (Centner et al., 2019; Douris et al, 2020).

Recent studies have reported that AT with BFR increases muscle strength, muscular hypertrophy, and physical function (Centner et al., 2019; Douris et al, 2020) and there are promises of positive effects on bone metabolism (Bittar, Pfeiffer, Santos & Cirilo-Sousa, 2018; Ferlito, Pecce, Oselame & De Marchi, 2020) in the older population. Low-load walk training with BFR has demonstrated improvements in the isokinetic knee extension (Abe et al, 2009) and flexion (Ozaki, Miyachi, Nakajima, and Abe, 2011; Ozaki et al, 2011), maximal dynamic strength (leg press and leg

curl) (Abe et al, 2009), muscle cross-sectional area (Abe et al, 2009; Ozaki et al., 2011), carotid arterial compliance (Ozaki, Miyachi, Nakajima & Abe, 2011), and functional ability (Abe et al, 2010; Clarkson, Conway & Warmington, 2017; Ozaki et al, 2011) of healthy older individuals. Despite these benefits, in some investigations, the application of BFR was short-term and controversial (Abe et al, 2009; Abe et al, 2010; Ozaki et al, 2011) since arbitrary pressures (not individualized) were used, which can be a serious methodological error, both affecting the results and increasing the risks to the health of the elderly. Therefore, AT associated to BFR may be more tolerable and relevant for people with limited muscle strength and/or bone fragility. Therefore, AT associated with RFS may be more tolerable and relevant for people with limited muscle strength and/or bone fragility (Howe et al, 2011). However, the chronic effects of AT with RFS on muscle activation and strength in elderly people (age > 60 years) with osteoporosis/osteopenia are poorly studied.

To fully understand the clinical practice of RFS to promote the benefits mentioned above and its relationships with bone health in older women, the present study aimed to apply TA combined with RFS (using individualized pressures) and analyze the effect of 24 weeks of AT with RFS on muscle activation and strength in overweight elderly women with osteopenia/osteoporosis. It is known that during aging, the body's ability to function decreases in a natural and physiological way (Cabral, S. Silva, Bispo, M. Silva, 2016), there is an increase in bone fragility, which results in serious physical losses with an increased risk of falls and fractures, psychological and social that often leave sequelae, making it impossible to carry out daily activities, leading the elderly to depend on others and frequent hospitalizations (Cabral, S. Silva, Bispo, M. Silva, 2016), thus increasing spending on public health, It is known that the number of public policies aimed at the elderly do not meet the growing demand of this population, a factor resulting from the increase in the life expectancy of the elderly in the Brazilian social context (Xavier, 2012), Therefore, our hypothesis is that low-intensity AT with RFS promotes an increase in muscle activation and strength similar to moderate-intensity AT and that the individualized RFS method can help different therapies to design more effective and tolerable training sessions and maximize adaptations in muscle activation and strength in elderly women with comorbidities, thus reducing public health spending and improving quality of life.

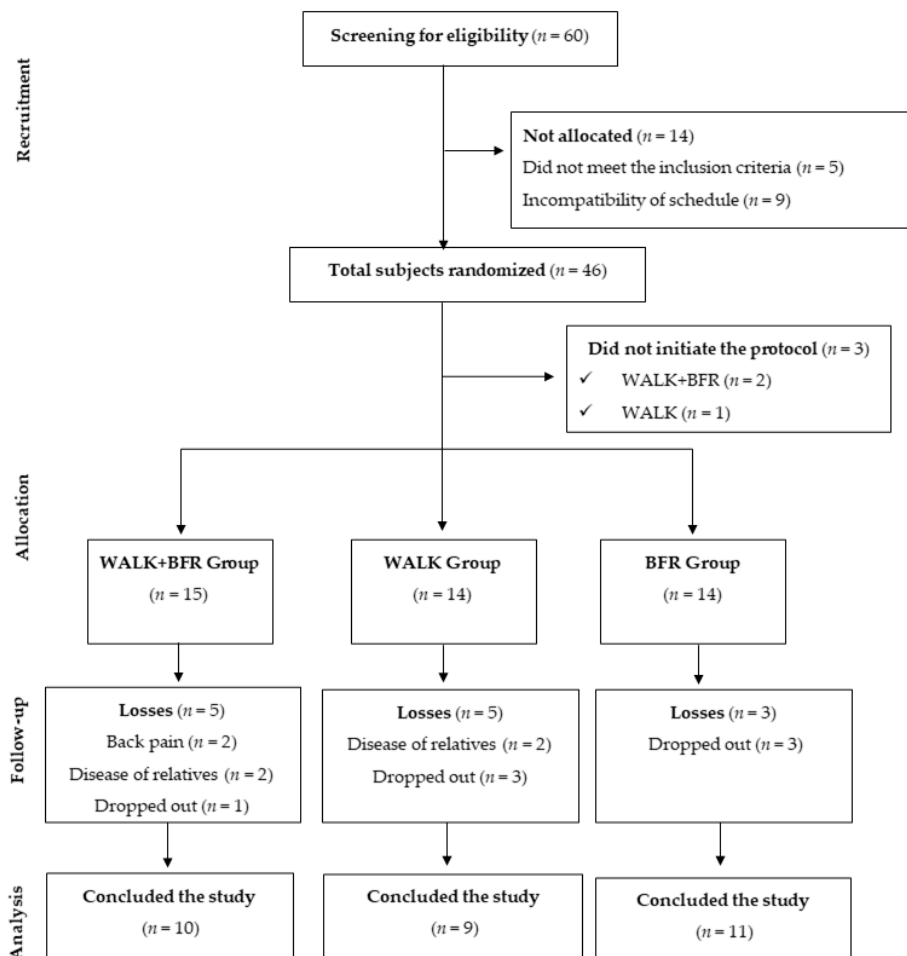
## 2. MATERIALS AND METHODS

### 2.1 Participants

Elderly women were selected using the following criteria: age  $\geq 60$  years; postmenopausal; with osteopenia (T-score [standard deviation] =  $-1.0$  to  $-2.5$ ) or osteoporosis (T-score [standard deviation]  $\leq -2.5$ ) in at least one of the areas analyzed by dual-energy X-ray absorptiometry (DXA) (Table 1); no hormone therapy in the last 3 months before the study; no AT and resistance training in the last 3 months before the study; irregularly active according to the International Physical Activity Questionnaire (walking frequently  $\leq 3$  times a week and duration  $\leq 30$  minutes or walking 4 times a week lasting  $\leq 20$  minutes and moderate physical activity once a week lasting  $\leq 30$  minutes); Ankle-brachial index between 0.91 and 1.30; no musculoskeletal or cardiorespiratory diseases; and no use of medications that could interfere on the bone metabolism (e.g. corticosteroids). Elderly

women with musculoskeletal pain or osteoarticular dysfunction during the intervention period, who attended fewer than 85% of the session, or who dropped out, were excluded.

A total of 60 women volunteered for the current study. However, five participants did not meet all the inclusion criteria, nine were not able to fit the study into their schedule, three requested to be withdrawn from the study before initiating the training, and 13 participants dropped out of study during training for personal reasons (Figure 1). Thus, 30 elderly women [age: 66 (5) years; body mass: 66.2 (10.7) kg; height: 1.52 (0.04) m; BMI: 28.7 (4.5) kg/m<sup>2</sup>] who were overweight (24.9 <BMI< 30 kg/m<sup>2</sup>) and had osteopenia/osteoporosis were enrolled in the trial (Table 1). The participants were asked to maintain their usual diet and daily activities during the intervention. However, no nutritional and lifestyle monitoring were performed. The participants were recruited from a physiotherapy clinic and an association of retirees of the local university. The study was conducted at João Pessoa-PB, in northeast Brazil.



**Figure 1. Sample recruitment flowchart. WALK+BFR, low-intensity walk with blood flow restriction intervention; WALK, moderate-intensity walking intervention; BFR, blood flow restriction intervention.**

A priori sample size was estimated using the G\*Power 3.1.9 software (Franz Faul, University Kiel, Germany) for an RM-ANOVA (within-between interaction), given  $\alpha = .05$ , power  $(1-\beta) = .8$ , and

effect size (ES)= .7 (large). Therefore, a minimum of 27 participants was required. A similar effect size has been observed in previous clinical trials involving the BFR approach with elderly women (Clarkson et al., 2017; Ozaki, Miyachi, Nakajima, and Abe, 2011; Ozaki et al, 2011). Since some volunteers denied or were unable to carry out the evaluations, the analyzes were performed by protocol.

This study was approved by the local Ethics Committee (CAAE: 67125317.1.0000.5188) following the Declaration of Helsinki and registered on the Brazilian Clinical Trials Registration Platform (RBR-3d957w). Informed consent was obtained from all individual participants included in the study.

**Table 1. Anthropometric, demographic, and Soné mineral density data of the participants**

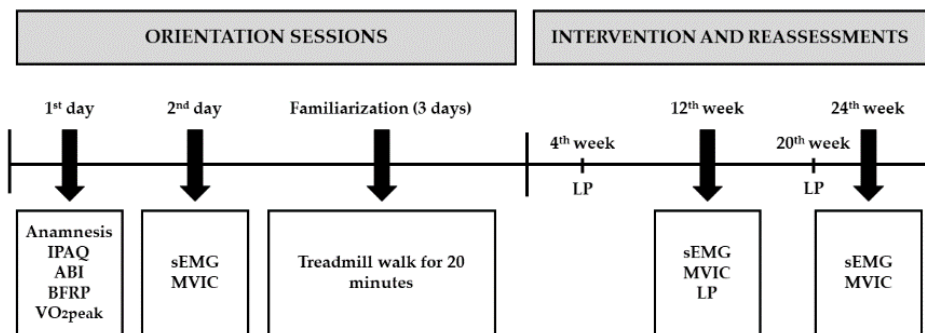
Variables	Total	WALK	WALK+BFR	BFR
	(n = 30)	Group (n = 9)	Group (n = 10)	Group (n = 11)
Age (years)	66.0 (4.6)	64.1 (2.5)	65.2 (5.3)	68.4 (4.7)
Height (m)	1.52 (0.04)	1.52 (0.04)	1.53 (0.05)	1.50 (0.03)
Body mass (kg)	66.2 (10.7)	64.7 (10.5)	69.4 (8.2)	64.9 (12.8)
Body mass index (kg/m <sup>2</sup> )	28.7 (4.5)	28.0 (4.1)	29.7 (3.3)	28.6 (5.7)
<i>Ethnicity</i>				
White	9 (30.0)	2 (22.2)	5 (50.0)	2 (18.2)
Black	10 (33.3)	2 (22.2)	5 (50.0)	3 (27.3)
Mixed	11 (36.7)	5 (55.6)	0 (0.0)	6 (54.5)

<i>Bone</i>	<i>Mineral</i>			
<i>Density</i>				
T-LS				
Normal	5 (16.7)	2 (22.2)	1 (10.0)	2 (18.2)
Osteopenia	16 (53.3)	5 (55.6)	6 (60.0)	5 (45.5)
Osteoporosis	9 (30.0)	2 (22.2)	3 (30.0)	4 (36.4)
T-FN				
Normal	6 (20.0)	2 (22.2)	2 (20.0)	2 (18.2)
Osteopenia	22 (73.3)	7 (77.8)	8 (80.0)	7 (63.6)
Osteoporosis	2 (6.7)	0 (0.0)	0 (0.0)	2 (18.2)
T-TF				
Normal	14 (46.7)	6 (66.7)	4 (40.0)	4 (36.4)
Osteopenia	14 (46.7)	3 (33.3)	5 (50.0)	6 (54.5)
Osteoporosis	2 (6.7)	0 (0.0)	1 (10.0)	1 (9.1)

**Note:** Continuous data are presented as mean (SD) and categorical data as absolute and relative (%) frequency. Abbreviations: WALK+BFR, low-intensity walk with blood flow restriction intervention; WALK, moderate-intensity walking intervention; BFR, blood flow restriction intervention; T-LS, total score lumbar spine; T-FN, total score femoral neck; T-TF, total score femur; BMI, body mass index.

## 2.2 Experimental design and procedures

A 3 (training group: WALK, WALK+BFR or BFR) 3×3 (time: baseline, 12, and 24 weeks) factorial randomized clinical trial was performed. On the first day, the elderly women performed ankle-brachial index, body composition by DXA (Lunar Prodigy Advance®, GE Healthcare, USA), BFR pressure, and maximum oxygen uptake (VO<sub>2peak</sub>) measures. On the second day, 48 hours after the first session, muscle activation and strength were recorded concomitantly, by electromyography and dynamometry, and from the third to the fifth day, three familiarization sessions were performed followed by the intervention. Outcomes were assessed at baseline, after 12 weeks, and 24 weeks (Figure 2). Participants were randomized (www.randomization.com) among the intervention groups before starting data collection. One of the researchers was blinded for the statistical analyses. All volunteers were instructed not to change their habits during the study (6 months), being free from dietary restrictions or any type of nutritional advice.



**Figure 2.** Study design. ABI, ankle-brachial index; BFRP, blood flow restriction pressure; IPAQ, International Physical Activity Questionnaire; VO<sub>2peak</sub>, maximum oxygen uptake; sEMG, surface electromyography; MVIC, maximum voluntary isometric contractions. LP, load progression (treadmill speed).

### 2.2.1 Maximal oxygen uptake

To estimate VO<sub>2peak</sub> (ml.kg<sup>-1</sup>.min<sup>-1</sup>) and ensure the speed progression on the treadmill (820EX, Embreex, Brazil) for the AT, a submaximal test was performed (Cirilo-Sousa et al, 2014). The test was interrupted when the volunteers reached 85% predicted maximum heart rate (210 – age), responding above 7 on the scale of perceived exertion for walking/running exercise (Borg 0-10), or voluntary withdrawal due to muscle fatigue or cyanotic appearance. The progression in the training load (treadmill speed) was performed at weeks 4, 12, and 20 (Figure 2).

### 2.2.2 Dynamometry

The maximum isometric strength of the knee was measured using a digital dynamometer (DD-300, Instrutherm Ltd., Brazil) with the participant sitting in an adapted Bonett chair, keeping the knee at a 60° angle for the extensors and a 30° angle for the flexors, measured by a fleximeter (Instituto Code de Pesquisa, Brazil), with the trunk supported on the backrest and stabilized by restraining belts on the trunk, pelvis, and thigh of the lower limb to be evaluated. During all the



test procedures, the participants were instructed to firmly hold the lateral supports of the seat to stabilize all body segments. The participants performed three sub-maximal repetitions 20 minutes before data collection to familiarize themselves with the procedures.

The test consisted of a serie of three maximum voluntary isometric contractions for 5 seconds, with 1min-interval between contractions, being that the mean of the 3 isometric contractions was recorded.

### 2.2.3 Electromyography

Surface electromyography (sEMG) of the flexor muscles (semitendinosus – ST and biceps femoris – BF) and extensor muscles (vastus medialis – VM and vastus lateralis – VL) of the knee were recorded using an electromyograph (W4X8, Biometrics Ltd., UK) with eight channels, Bluetooth, and the following technical specifications: hardware with a 12-bit analog-to-digital conversion card; amplifier with a gain of 1000x; 20 to 500 Hz bandpass filter (second-order Butterworth); common-mode rejection ratio > 100 dB; signal noise ratio < 3  $\mu$ V root mean square; 109 Ohm impedance; superficial, bipolar, active, differential simple electrodes, with preamplification of 20x; and a sampling frequency of 1000 Hz. After shaving, cleaning the skin, and marking the points with henna dye, the electrodes were fixed on the ST, BF, VL, and VM muscles, according to Surface Electromyography for the Non-Invasive Assessment of Muscles – SENIAM (Hermens, Freriks, Disselhorst-Klug & Rau, 2000), while the reference electrode was fixed on the lateral malleolus of the contralateral limb, always by the same examiner. The mean square root was used to process the signal from the central 3 seconds of the signal window during the 5 seconds of maximum voluntary isometric contraction. The reference value for normalization of the sEMG signal was the maximum voluntary isometric contraction peak of each muscle, and to ensure the comparison between subjects and groups, the VL+VM and BF+ST values were summed. Both for recording the strength and for sEMG, randomization was performed between the lower limbs ([www.randomization.com](http://www.randomization.com)).

### 2.2.4 Blood flow restriction (evaluation and intervention)

The total arterial limb occlusive pressure (LOP) was measured using vascular Doppler (MedPeg® DV-2001, Ribeirão Preto, SP, Brazil). The probe was positioned over the posterior or pedial tibial artery to determine the arterial pressure (mmHg) of the lower limb. The volunteers remained in the supine position and a standard blood pressure sphygmomanometer for obese patients (width 180 mm; length 900 mm) was fixed in the region of the inguinal fold and inflated to the point where the auscultatory pulse of the tibial artery was interrupted according to the study by Laurentino et al. (2012). The LOP was measured at the baseline, and after 12 and 24 weeks of training, in order to check possible morphological adaptations (i.e, increased muscle mass) that could influence in LOP values.

For WALK+BFR and BFR groups, pneumatic tourniquet cuffs were placed on the proximal portions of the thighs and remained inflated throughout the session (Laurentino et al, 2012). In general, the BFR pressure protocol (cuff pressure) was applied as follows: familiarization and on



1st month [20% LOP= 35.2 (5.3) mmHg]; 2nd month [30% LOP= 52.5 (8.1) mmHg]; 3rd month [40% LOP= 65.6 (12.4) mmHg]; and 4th to 6th month [50% LOP= 82.4 (13.8) mmHg]. Because it is a long-term intervention (24 weeks) with the blood flow restriction method compared to other studies with the elderly (Abe et al, 2010), we chose to increase the restrictive pressure monthly until the maximum could reach 50% (5th and 6th month) of the BFR pressure, since the literature has presented evidences that 50% of the BFR promotes similar effects to 80% of BFR and it does not seem to promote any damage to health or discomfort (Loenneke, Thiebaud, Abe & Bembem, 2014).

Before each training session, the women performed a stretching the upper limbs for 10 minutes with emphasis on the antero-internal chain, the posterior chain, and the extensor muscles of the shoulders and trunk. Then, a stretching was performed for the lower limbs for the knee extensor and flexor muscles. Finally, the participants performed one of three training protocols – with progression of speed for the AT, which they were prescribed according to the VO<sub>2</sub>peak test as follow: (1) WALK: The participants performed 20 minutes of moderate-intensity walking on an ergometric treadmill at 60% VO<sub>2</sub>peak, (2) WALK+BFR: The participants performed 20 minutes of low-intensity walk on an ergometric treadmill at 40% VO<sub>2</sub>peak combined with BFR, (3) BFR: The participants, lying in the supine position, performed 20 minutes of BFR. All intervention protocols were performed 3 times per week (Monday, Wednesday, and Friday) for 6 months. Table 2 summarizes the mean of HR response and cuff pressure in different percentage of LOP applied during the intervention.

**Table 2. Heart rate (HR) response and cuff pressure in different percentage of total arterial limb occlusive pressure (LOP) (right and left) of the training sessions during the intervention.**

Time	Variable	Group		
		WALK	WALK+BFR	BFR
	HR (bpm)	104 (8)	98 (8)	73 (8)
1 <sup>st</sup> month	20% LOP right (mmHg)		34.3 (4.6)	36.5 (6.1)
	20% LOP left (mmHg)		33.4 (5.8)	36.5 (7.6)
2 <sup>nd</sup> month	HR (bpm)	102 (7)	96 (7)	71 (7)

	30% LOP right (mmHg)		51.4 (6.9)	54.7 (9.1)
	30% LOP left (mmHg)		50.1 (8.7)	54.7 (11.3)
	HR (bpm)	101 (5)	97 (9)	71 (8)
3 <sup>rd</sup> month	40% LOP right (mmHg)		68.5 (9.1)	72.9 (12.1)
	40% LOP left (mmHg)		66.8 (11.6)	72.9 (15.1)
	HR (bpm)	101 (6)	98 (8)	71 (8)
4 <sup>th</sup> month	50% LOP right (mmHg)		85.6 (11.4)	91.1 (15.1)
	50% LOP left (mmHg)		83.5 (14.5)	91.1 (18.9)
	HR (bpm)	100 (5)	98 (8)	70 (8)
5 <sup>th</sup> month	50% LOP right (mmHg)		85.6 (11.4)	91.1 (15.1)
	50% LOP left (mmHg)		83.5 (14.5)	91.1 (18.9)
	HR (bpm)	100 (3)	98 (6_)	68 (7)
6 <sup>th</sup> month	50% LOP right (mmHg)		85.6 (11.4)	91.1 (15.1)
	50% LOP left (mmHg)		83.5 (14.5)	91.1 (18.9)

**Note:** Data presented by mean (SD).

### 2.3 Statistical Analysis

Multiple imputations of missing data from the outcomes were performed for sEMG variables with missing values in the evaluations after 12 weeks. Data presented normal distribution (Shapiro-Francia test) and they are reported by mean and standard error (SEM). Data were analyzed using two-way repeated-measures ANOVA (3 training conditions  $\times$  3 times). If a significant group, time, or interaction effect was observed, post hoc analyses were performed by the unequal HSD post hoc (Tukey-Kramer method). Levene's test confirmed that the error variance of the dependent variables was equal across groups. Mauchly's test was used to test the assumption of sphericity, and when it was violated, the Greenhouse-Geisser corrected values were used. Moreover, effect size (ES) was estimated by the Cohen's  $d$  (pretest to posttest designs and pre-post-control design), in which WALK was considered as reference, using the psychometrica calculator and interpreted as:  $d < 0.20$  trivial,  $d = 0.20-0.59$  small,  $d = 0.60-1.19$  moderate,  $d = 1.20-1.99$  large,  $d = 2.00-3.99$  very large and  $d \geq 4.0$  almost perfect (Hopkins, Marshall, Batterham & Hanin, 2009). To assess the correlation between muscle activation and strength of the knee flexors and extensors at baseline, 12, and 24 weeks of intervention, Pearson's correlation test was used. Data were analyzed using the TIBCO Statistica version 14 (TIBCO Software Inc., USA) and the graphs were build using GraphPad Prism 8 (GraphPad software, LLC, USA). The results at  $P < .05$  were considered statistically significant.

### 3 RESULTS

The comparison of the muscle activation (sEMG) and strength for knee flexors and extensors over the 24 weeks of intervention in overweight elderly women with osteopenia/osteoporosis are summarized in Figure 3. There were no significant group differences for any variable at baseline ( $P > .05$ ).

#### 3.1 Surface electromyography of the knee flexors and extensors muscles

A significant main effect was found for time in the sEMG of the knee flexors ( $F_{2, 54} = 12.7$ ,  $P = .001$ ) and extensors ( $F_{2, 54} = 47.7$ ,  $P = .001$ ). Moreover, a significant training condition  $\times$  time interaction was only found in the sEMG of the knee extensors ( $F_{4, 54} = 3.4$ ,  $P = .014$ ). Post hoc analysis revealed that all groups improved the muscle activation of the knee extensors at 24 weeks in a similar manner, but only the BFR group increased the muscle activation at 12 weeks in comparison to baseline (Figure 3).

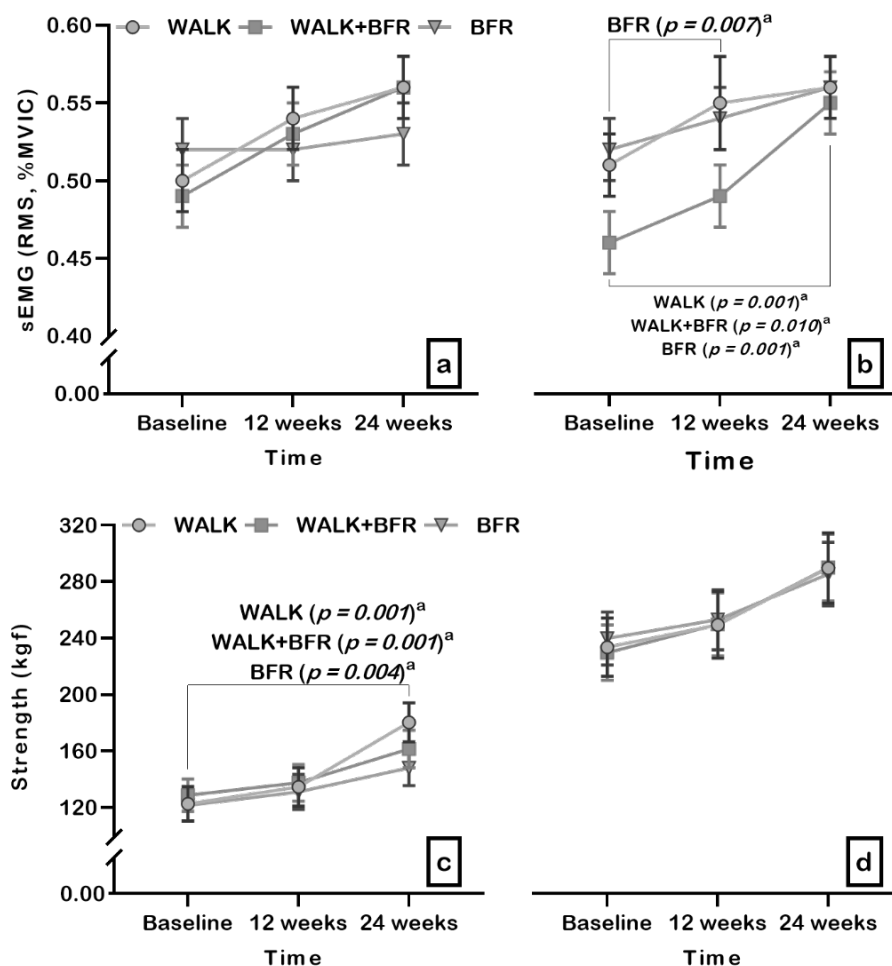
From the ES analysis, the WALK+BFR group presented ESs small and moderate for sEMG, respectively at 12 weeks ( $d = .59$ ) and 24 weeks ( $d = .95$ ) for the knee flexors. For the knee extensors, trivial and adverse ESs were found for WALK+BFR at 12 weeks ( $d = .16$ ) and 24 weeks ( $d = -.19$ ), respectively.

Moreover, the BFR group presented ESs small and moderate, respectively at 12 weeks ( $d = .52$ ) and 24 weeks ( $d = .99$ ) for the knee flexors. For the knee extensors, small ESs were only found for BFR at 12 weeks ( $d = .28$ ) and 24 weeks ( $d = .52$ ), respectively.

#### 3.2 Strength of the knee flexor and extensor muscles

A significant main effect was found for time in the strength of the knee flexors ( $F_{2, 54} = 52.5, P = .001$ ) and extensors ( $F_{1.6, 43.6} = 43.6, P = .001$ ). Moreover, a significant training condition  $\times$  time interaction was only found in the strength of the knee flexors ( $F_{4, 54} = 3.4, P = .015$ ). Post hoc analysis revealed that all groups showed a similar improvement on the strength of the knee flexors at 24 weeks (Figure 3).

From the ES analysis, the WALK+BFR group presented ESs at 12 weeks ( $d = -.09$ ) and 24 weeks ( $d = -.66$ ) for the strength of the knee flexors. For the knee extensors, only trivial ESs were found for WALK+BFR at 12 weeks ( $d = .07$ ) and 24 weeks ( $d = .07$ ), respectively. Additionally, the BFR group presented ESs, respectively at 12 weeks ( $d = -.06$ ) and 24 weeks ( $d = -.80$ ) for the knee flexors. For the knee extensors, ESs were found for BFR at 12 weeks ( $d = -.04$ ) and 24 weeks ( $d = -.16$ ), respectively.



**Figure 3. Muscle activation (sEMG) [Panel a and b] and muscle strength [Panel c and d] for knee flexors (left side) and extensors (right side), respectively, over the 24 weeks of intervention in older women with osteopenia or osteoporosis. Data presented by estimated mean and SEM. aPost hoc intragroup difference (time),  $P < .05$ . WALK, moderate-intensity walking intervention. WALK+BFR, low intensity walks with blood flow restriction intervention. BFR, blood flow restriction intervention.**

### 3.3 Correlations between the strength and surface electromyography

The correlations between the strength and sEMG of the knee flexors and extensors are summarized in table 3. The relationship pattern was different for muscle and training groups.

**Table 3. Pearson's correlation (r) between muscle activation and strength for knee flexors and extensors of the overweighted elderly women with osteopenia or osteoporosis.**

Variable	Time	Training group		
		WALK	WALK+BFR	BFR
Knee flexors	Baseline	0.76 [0.19; 0.94]	-0.22 [-0.75; 0.47]	0.20 [-0.46; 0.71]
	12 weeks	0.74 [0.15; 0.94]	0.28 [-0.42; 0.77]	0.36 [-0.31; 0.79]
	24 weeks	0.48 [-0.27; 0.86]	0.67 [0.10; 0.91]	0.19 [-0.46; 0.71]
Knee extensors	Baseline	0.31 [-0.44; 0.80]	-0.14 [-0.71; 0.53]	-0.71 [-0.92; -0.20]
	12 weeks	0.54 [-0.18; 0.88]	-0.25 [-0.76; 0.45]	-0.70 [-0.91; -0.18]
	24 weeks	0.46 [-0.29; 0.86]	-0.28 [-0.77; 0.42]	-0.63 [-0.89; -0.04]

**Note:** Data reported by r and [95% CI].

Abbreviations: WALK+BFR, low-intensity walk with blood flow restriction intervention; WALK, moderate-intensity walking intervention; BFR, blood flow restriction intervention.

## 4 DISCUSSION

This randomized clinical trial combined AT with BFR using individualized pressures and analyzed the chronic effects on muscle activation and strength of the knee flexors and extensors in elderly women. Our novel observation is that both low-intensity AT with BFR and BFR alone over

24 weeks elicited a greater muscle activation of the knee flexors in comparison with AT exclusively. However, the strength gain of knee flexors was similar among the interventions at 24 weeks. Therefore, our hypothesis that low-intensity AT with BFR would promote an increase in muscle activation and strength similar to moderate-intensity AT was partially rejected.

The present investigation reinforces the use of BFR as an effective, tolerable, and potential clinical rehabilitation approach (Centner et al., 2019; Hughes et al, 2017). In our study, this statement is supported by the neuromuscular adaptations of the knee flexors (sEMG: +13%; strength: +22%) and extensors (sEMG: +18%; strength: +19%) after 24 weeks only using individualized pressures. Despite that, our statistical analysis has indicated that the activation of the selected muscles seemed to be more susceptible to improvement than the strength gain in overweight elderly women with osteopenia/osteoporosis. Thus, older women, for whom a conventional exercise program might be limited due to comorbidities, high mechanical stress to bones and joints, can perform this alternative BFR method (Centner et al., 2019).

Interventions to reduce the loss of strength and muscle activation seem to decrease the aging-induced risk of falls and fractures, in which the risk of some non-spine fractures is higher for older adults with overweight/obesity (Kelly, Gilman, Boschiero & Ilich, 2019; Scott et al, 2016). Aerobic or resistance exercises combined with the BFR technique are recommended to mitigate musculoskeletal damage (Hackney, Brown, Stone & Tennent, 2018; Loenneke et al, 2012). It is important to emphasize that the failure in muscle activation creates a barrier to the benefit of strength training, as it generates the inability to voluntarily contract the muscle, impairing the function (Hackney et al., 2018). According to Petterson et al. (2008), changes in quadriceps activation are directly related to quadriceps muscle strength in knee joint degeneration, whereas loss of lean mass appears to be a secondary mechanism of weakness in patients with osteoarthritis.

For activation of knee flexors, a moderate effect in favor of AT with BFR and only BFR was found compared to moderate-intensity AT. Although the BFR method induces more intense acute muscle activation compared to a control condition (Counts et al, 2016; Yasuda et al, 2009), evidence is scarce on the long-term effects of an AT with RFS on muscle activation in the elderly. In the present study, one of the factors that may explain the differences is based on the low RFS pressure, with its progressive increase over a period of 24 weeks, since a higher percentage of the estimated arterial occlusion pressure seems to provide the muscular response more robust (e.g. muscle activation, torque, muscle strength) (Abe et al., 2010; Ozaki, Miyachi, Nakajima, and Abe, 2011; Ozaki et al., 2011). However, some of these studies that applied low-intensity AT did not individualize the BFR pressures for the older participants (Ozaki et al., 2011).

The reduction in oxygen supply and the accumulation of metabolites induced by BFR might be responsible for the participation of higher threshold motor units even at low intensities (Yasuda et al., 2009). Additionally, studies suggest the BFR method induces a significant increase in the recruitment of fast-twitch fibers for maintenance and preservation of strength production during the performance of the exercise (Yasuda et al., 2009). On the other hand, a higher EMG amplitude might not necessarily represent a higher motor unit recruitment and hence our results must be interpreted with caution. Several intrinsic and extrinsic factors also affect the EMG signal. For example, if the motor unit newly recruited with the training is located close to the electrode, the EMG signal will be greater than the corresponding strength increase. Therefore, the non-linear

relationship causes the amplitude of the EMG signal to increase more than muscle strength (De Luca, 1997).

Regarding the strength of the knee extensors, this investigation is supported by similar studies (Ozaki, Miyachi, Nakajima, and Abe, 2011; Ozaki et al, 2011). With analogous training protocols, Ozaki, Miyachi, Nakajima, and Abe (2011) and Ozaki et al. (2011) did not observe a statistically significant increase in isokinetic and isometric knee extension, respectively in healthy older individuals. Other training protocols have also shown no advantage in combining the exercise with BFR for increasing muscle strength in older adults with osteoarthritis (Libardi et al, 2015). To our knowledge, this is the first chronic study ( $\geq 16$  weeks) analyzing overweight elderly women with osteopenia/osteoporosis and there is no strong evidence demonstrating strength gains employing AT with BFR.

Despite the individualized prescription of BFR training, the strength gains of the knee flexors muscle was similar to observed in the control walking group. This fact contrasts with previous randomized clinical trials, in which a treadmill walking with BFR training for 10 weeks (20 min  $\times$  4 days/week) increased the knee flexors strength about 15% for older adults (Ozaki, Miyachi, Nakajima & Abe, 2011) and 22% for healthy older women (Ozaki et al, 2011). Besides, Clarkson, Conway, and Warmington (2017) observed a greater increase in strength using the sit-to-stand test in older adults who performed walking with BFR (10 min  $\times$  4 days/week) for 6 weeks. Such differences may be explained by the higher cuff pressure applied in these studies ( $\geq 60\%$  LOP or  $\geq 140$ mmHg). From a practical standpoint, our results indicate that low-intensity AT with BFR does not seem to have long-term advantages on strength gain for the knee flexors in comparison to moderate-intensity walking. However, there is insufficient evidence focusing on the physiological mechanisms.

The correlation pattern between muscle activation and strength was different between groups, but inconsistent for most of them. In the CAM+RFS group, the magnitude of this relationship increased over time for the knee extensors. However, despite the moderate correlation at 24 weeks, this did not result in greater strength gains when compared to the no-RFS group. These results are in line with the study by Lixandrao et al (2018), which highlights that based on resistance training and RFS approach, RFS pressure does not influence the magnitude of strength gains. Therefore, the increase in strength throughout the intervention for both groups seems to be more influenced by the progression of walking speed than by RFS pressure.

This study provides the first reference for chronic effects longer than 16 weeks, applying individualized BFR pressure and continuous load progression in older people with comorbidities. Our data, obtained from overweight elderly women with osteopenia/osteoporosis, demonstrated the relevant role of the BFR method to promote long-term muscle activation. Moreover, the present purpose supports that lower and more tolerable cuff pressures may elicit sufficient long-term musculoskeletal adaptations while minimizing the risk of adverse events and discomfort, highlighting the need for individualized prescription of BFR training. Although some evidence supports the positive effects of exercise with BFR on bone metabolism (Ferlito, Pecce, Oselame & De Marchi, 2020; Kanazawa et al., 2009), the presence of chronic disease, sedentary behavior, and/or insufficient physical activity in older women are factors that need further investigation in the areas of BFR and physical exercise. The evasion of 13 participants, the lack of an experimental



group to perform the low-intensity AT without the RFS and an experimental group with arbitrary pressure, can be listed as limitations of the study, which reduced the possibility of finding a more significant effect for the studied variables.

## 5 CONCLUSION

In conclusion, the BFR-based interventions presented similar strength gains for the knee flexors in comparison to moderate-intensity walking training. However, overweight elderly women with osteopenia/osteoporosis may benefit from using analogous strategies to achieve long-term neuromuscular gains due to increased activation of the knee extensors over 24 weeks. In addition, we reiterate the isolated application of BFR is an alternative training, with an effective, tolerable, and potential clinical focus on the approach to rehabilitation of older women.

### Disclosure statement

The authors report there are no competing interests to declare.

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