Effect of walking with blood flow restriction in elderly women with osteoporosis/osteopenia

Efeito da caminhada com restrição do fluxo sanguíneo em idosas com osteoporose/osteopenia

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Date of first submission: February 7, 2023
Last received: May 23, 2023
Accepted: May 24, 2023

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Abstract

Introduction: The preservation of bone mass in elderly women is associated with better levels of practice of systematic physical exercises. Aerobic training combined with blood flow restriction seems to be a new alternative that determines this process, but knowledge gaps are still observed when referring to exercise associated with blood flow restriction (BFR) and adaptations on bone variables. **Objective:** To analyze the chronic effects of aerobic training with and without BFR on bone mineral density and bone biomarker osteocalcin concentrations in older women. **Methods:** Thirty women were randomized into the following groups: walking on a treadmill at low intensity with BFR; moderate treadmill walking with no BFR; only BFR (no exercise) for 20 minutes, twice a week, for 24 weeks. Bone mineral density was measured before and 24 weeks after intervention. Blood serum osteocalcin concentrations were measured before, 12 and 24 weeks after intervention. **Results:** There were no differences between groups in bone mineral density (femoral neck, $p = 0.31$; total femur, $p = 0.17$; lumbar spin, $p = 0.06$) and osteocalcine ($W(2) = 0.27$; $p = 0.87$) ouctomes after 24 weeks of intervention. **Conclusion:** There was no difference between walking training, blood flow restriction only, or walking+blood flow restriction on bone mineral density and osteocalcin concentrations after 24-weeks of intervention in older women with osteopenia/osteoporosis.

Keywords: Aging. Bone mineral density. Exercise. Osteocalcin. Therapeutic occlusion.

Resumo

Introdução: *A preservação da massa óssea em mulheres idosas está associada a melhores níveis de prática de exercícios físicos sistemáticos. O treinamento aeróbico combinado com restrição de fluxo sanguíneo (RFS) parece ser uma nova alternativa que determina esse processo, mas ainda são observadas lacunas de conhecimento quando se refere ao exercício associado à RFS e adaptações nas variáveis ósseas.* **Objetivo:** *Analisar os efeitos crônicos do treinamento aeróbico com e sem RFS na densidade mineral óssea e nas concentrações do biomarcador ósseo osteocalcina em mulheres idosas.* **Métodos:** *Trinta mulheres foram randomizadas nos seguintes grupos: caminhada em esteira de baixa intensidade com RFS; caminhada moderada em esteira sem RFS; apenas RFS (sem exercícios) por 20 minutos, duas vezes por semana, durante 24 semanas. A densidade mineral óssea foi medida antes e 24 semanas após a intervenção. As concentrações séricas de osteocalcina no sangue foram medidas antes, 12 e 24 semanas após a intervenção.* **Resultados:** *Não houve diferenças entre os grupos na densidade mineral óssea (colo do fêmur, p = 0,31; fêmur total, p = 0,17; giro lombar, p = 0,06) e osteocalcina (W(2) = 0,27; p = 0,87) após 24 semanas de intervenção.* **Conclusão:** *Não houve diferença entre treinamento de caminhada, apenas restrição de fluxo sanguíneo ou caminhada + restrição de fluxo sanguíneo na densidade mineral óssea e nas concentrações de osteocalcina após 24 semanas de intervenção em mulheres idosas com osteopenia/osteoporose.*

Palavras-chave: *Envelhecimento. Densidade mineral óssea. Exercício. Osteocalcina. Oclusão terapêutica.*

Introduction

The reduction of estrogen production, characteristic of the post-menopausal life stage, accelerates the reduction in bone mineral density (BMD) that makes women more susceptible to postmenopausal osteoporosis and to an increased risk for fracture. This fact constitutes a public health problem, with high rates of morbidity and mortality in the older people.[1]

The analysis of bone biomarkers contributes to earlier information about bone remodeling compared to the changes observed from a bone densitometry scan. As a result, biochemical markers are more sensitive to the effects of physical exercise on bone formation and resorption in both acute or chronic training protocols and are therefore considered more effective to examine the effects of exercise on bone remodeling.[2,3]

The American College of Sports Medicine recommends several types of exercise for the preservation of bone health in adulthood, especially in women after menopause.[4] Several studies have observed the effect of different exercises (aerobic, high impact, strength),[5-7]or their combination, on BMD in elderly women, however, bone mass gains are less than 2% for the lumbar spine.[8] Aerobic training, without any other intervention, has little or no effect on BMD of the spine and the femoral neck. In a meta-analysis, Palombaro[9] recommends walking, or any low impact exercise, combined with other forms of training to preserve bone mass in women after menopause. The implementation of high intensity exercise programs is not feasible in some situations, for instance for older persons with joint restrictions such as osteoarthritis, herniated discs, and vertebral fractures.[10] Thus, much of the scientific community has been looking for alternatives that use low intensity exercises for such individuals to improve bone health.

In this perspective, in order to reduce load-related stress, the Japanese, nearly 50 years ago, developed a method of strength training called Kaatsu training or blood flow restriction (BFR) training that consists of using low loads (20 to 30% of 1RM) combined with BFR promoted by means of elastic bands or standard sphygmomanometers.[5,11]

The neuromuscular benefits of the BFR technique combined with strength training[12] and aerobic training[13,14] are well described in the literature. However, scarce studies investigated bone health following BFR exercise. According to Bittar et al.,[15] only one study observed a 10.8% increase in the bone-specific alkaline phosphatase biomarker following aerobic training when combined with BFR compared to an aerobic training group only, after a three-week intervention with young adults.[16] However, no studies were found that evaluated the chronic effect of this technique on the BMD and its biomarkers in older individuals. Thus, the present study aimed to analyze the chronic effects of aerobic training combined with BFR on the bone health of older women with osteopenia or osteoporosis.

Methods

Participants

Thirty women aged from 60 to 76 years, with osteopenia or a confirmed diagnose of osteoporosis volunteered for the current study. This investigation included only individuals that met all of the following inclusion citeria: 1) osteopenia (t score between -1.0 and -2.5 standard deviations) or osteoporosis (t score < -2.5 standard deviations) in the lumbar spine (L1 to L4), femoral neck, or total femur regions; 2) postmenopausal, with a minimum 12 months period since the last menstrual period;[17] 3) no hormonal replacement therapy in the last three months; 4) no involvement in any resistance or aerobic training programs during the previous three months; 5) insufficiently active and functionally independent, according to the extended form of the international physical activity questionnaire (IPAQ), that shows the following levels of physical activity: walking frequently ≤ 3 times a week and duration ≤ 30 minutes or walking 4 times a week lasting ≤ 20 minutes and moderate physical activity once a week lasting ≤ 30 minutes;[18] 6) have an ankle-brachial index between 0.91 and 1.30;[19] 7) free from any musculoskeletal or joint disease that could limit exercise performance; 8) not taking any medicines that could affect bone metabolism, 9) non-smokers; and 10) not consuming alcohol.

The ankle-brachial index (ABI) was measured as a safety criterion to determine the risk for obstructive arterial diseases of the lower limbs. Systolic pressures in the lower limbs were measured at the ankle (tibial posterior or pedial artery) and upper limbs (brachial artery), using a portable vascular Doppler (MedPeg® DV-2001, Ribeirão Preto, SP, Brazil). Subsequently, the index was calculated for each leg and consisted of the ratio between ankle systolic blood pressure and brachial systolic blood pressure.[19]

Participants were excluded from the study if: 1) were unable to achieve a minimum training frequency of at least 85%; 2) were injured; or 3) if they requested to be withdrawn from the study. Figure 1 outlines all the procedures involved in the screening process at the study entry. Sample size was determined a priori using G*Power (ES = 0.25, Power = 0.80, α = 0.05, three groups, and three measurements) following the recommendations from Faul et al.[20] and Beck.[21] According to our calculations, a total sample size of 36 individuals would be required for this study. 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 follow the training schedules, three requested to be withdrawn from the study before initiating the training, and thirteem during training for reasons unrelated to the study procedures, leaving a final sample size of 30 participants (Figure 1).

This study was approved by the Research Ethics Committee of the Health Sciences Center of the Universidade Federal da Paraíba (No. 2086608; CAAE: 67125317.1.0000.5188) and registered in the Brazilian Clinical Trials Registry Platform (RBR-3d957w). All volunteers signed an informed consent form.

Experimental design

This study was a prospective clinical trial that randomly allocated participants into one of the following experimental groups: 1) a walking group (W), which walked on a treadmill for 20 minutes at 60% of VO_2max; 2) a walking with blood flow restriction group (W+BFR), which performed 20 minutes of walking on a treadmill at 40% of VO_2max; and 3) a blood flow restriction group (BFR), which did not perform any exercise and only received the BFR stimuli. Participants were required to visit the laboratory for a total of 72 different occasions over the course of six months. During the first visit, participants were provided with an explanation about the study procedures, filled out standardized questionnaires, and had their ABI assessed. If the participants' ABI was within the range stated in the inclusion criteria, participants completed the next steps, which consisted of determing the total arterial occlusion pressure for the lower-body and an estimated peak oxygen consumption (VO_2peak) test. After a period of 24 hours, body weight and height were assessed and participants completed a total body dual x-ray absorptiometry (DXA) scan, followed by a blood draw, used to determine the plasm osteocalcin baseline levels. During the third visit, participants in the W and W+BFR groups were familiarized with the training protocols. Following the first 12 weeks of training, osteocalcin was assessed and BFR training pressures were adjusted. After 24 weeks, the same analysis was completed as done after week 12, with the addition of the DXA scans (Figure 2).

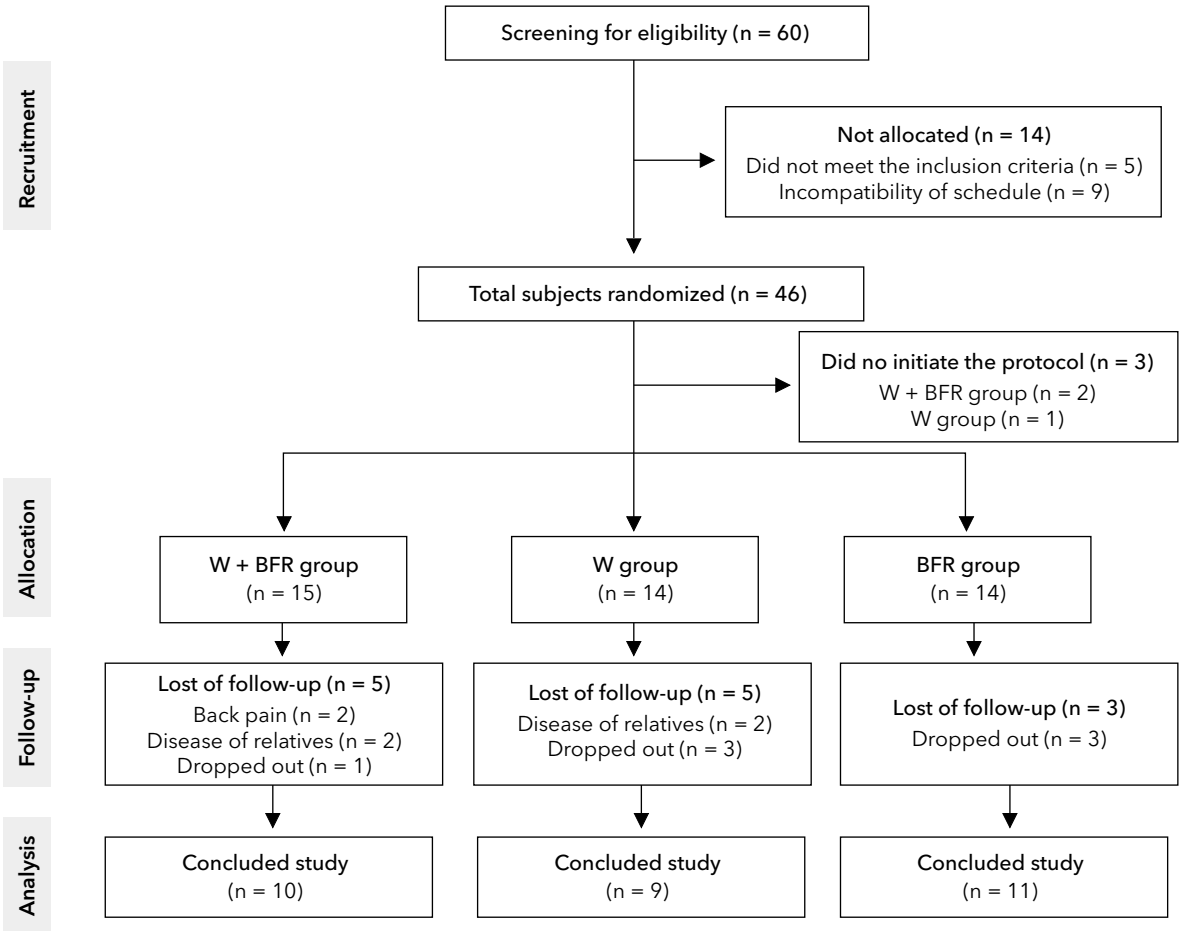

Figure 1 - Flowchart of subject recruitment and drop-out.

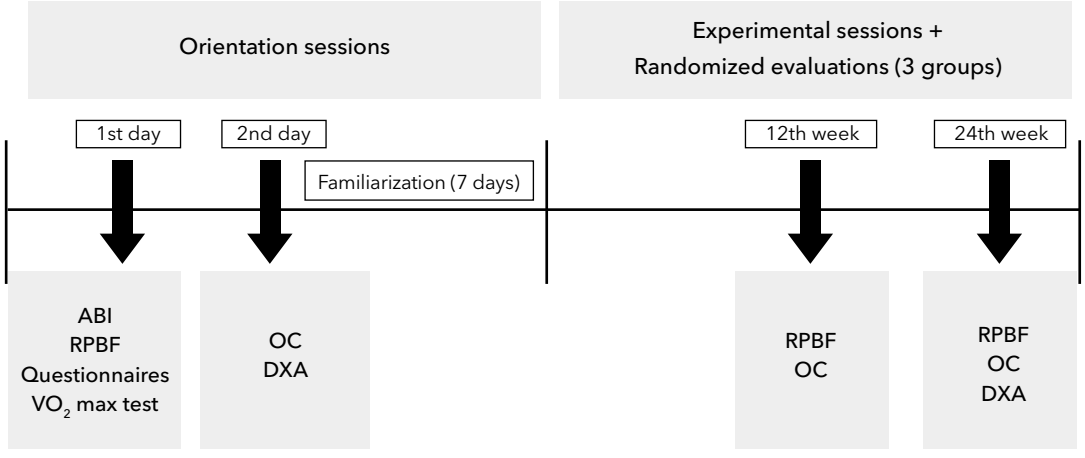

Figure 2 - Study design.

Note: ABI = ankle-brachial index; RPBF = restriction pressure of blood flow; VO_2 max = maximal oxygen consumption; OC = osteocalcin; DXA = dual x-ray absorptiometry.

Measurements

The pressure required to fully occlude blood flow to the lower limb was determined before training, following 12 weeks of training, and 24 weeks of training. Total BFR was assessed using an 18 cm wide cuff positioned below the inguinal crease and a handheld Doppler probe (MedPeg® DV-2001, Ribeirão Preto, SP, Brazil) placed over the posterior tibial or anterior tibial artery and it was used to detect the auscultatory pulse. The cuff was manually inflated continually until the point at which the auscultatory pulse could no longer be detected by the Doppler. This was considered the total occlusion pressure and used to determine the occlusion pressure during training. The same procedure was repeated in the contralateral limb.

The BFR method is considered safe since low (20 to 30%) to moderate (50%) arterial restriction pressures have resulted in significant physiological improvements without the need for high pressures which could lead to increased health risks.[22]

All volunteers recieved BMD scans at a specialized clinic, using Lunar-GER PRODIGY equipment (GE Medical Systems Lunar, Madison, WI, USA), by the same evaluator: lumbar spine (LS) images (L1/L4), right proximal femur scans of the femoral neck - FN) and total femur scans (TF), before and after 24 weeks of the intervention program. The minimum significant variation for LS and TS to be considered significant was 0.03 g/cm^2, and 0.035 g/cm^2 for FN.[23]

The adapted Cirilo bench step protocol was used with manual increments in the height of the steps according to the height of each volunteer, in order to estimate VO_2peak (ml/kg/min) indirectly and to establish the training speeds on the treadmill.[24]

The W+BFR group trained with a low load (40% of VO_2 max.) and the W group trained with a moderate load (60% of VO_2 max.). This test was performed pre-training, and at 1, 3, 5 and 6 months (end of the training intervention). Since the volunteers were classified as untrained, the test rhythm used was 116 steps per minute, as measured by a metronome (Tagima®, Japan).

Osteocalcin serum concentrations were assessed before training, following 12 weeks of training, and at the end of 24 weeks of training. Venous blood samples were collected by a nurse at 7am (±1), following an eight hour fasting period. Blood samples were allowed to clot at room temperature, then centrifuged. The plasma was separated and transferred to polystyrene tubes and frozen at -80 °C until the final analysis. All blood samples were analyzed by the Hermes Pardini Laboratory (Vespasiano, MG, Brazil). The serum concentration of osteocalcin was assessed using the Elecsys reagent, according to a protocol (EP5 A2) from the Clinical and Laboratory Standards Institute (CLSI), on the Modular analytics E170 (ROCHE) equipment. The sensitivity of the assay used was 0.5 to 300 ng/mL. The intra and inter assay coefficient of variation for this analysis were 4.76% and 8.00%, respectively.

Training protocols

The W+ BFR group trained with low load (40% of VO2 peak) and the W group trained with a moderate load (60% of VO_2 peak.). The BFR group was subjected to BFR without performing any type of exercise, twice a week, for 20 minutes, for six months. Volunteers in the W+BFR and BFR group used inflated pressure tourniquets on both proximal portions of the thighs, and throughout each 20 minute training session.[25] The cuff pressure used during the firs month of training was 20% of total occlusion pressure (TOP), and was then incrementally increased at two months (30% TOP), three months (40% TOP), and then remained the same pressure the in the remainder of the intervention at 50% TOP.[22]

Statistical analyses

All data were analyzed using the Statistical Package for the Social Sciences (SPSS - 20.0, IBM, New York) and the results plotted in GraphPad Prism software (5.03). Initially, data normality (Shapiro-Wilk test) and homogeneity of variances (Levene test) were verified. The performance of the groups, over time, for the variables BMD (LS, FN and TF) and osteocalcin were analyzed using Generalized Estimated Equations (GEE) considering the autoregressive covariance matrix (AR-1) and the link log function with gamma distribution model.

The selection of models for bone variables was based on Quase Likehood Independence Criterion – QIC.[26] The normality of the residues was verified using Q-Q charts and considered plausible in each instance. The Bonferroni post hoc test was used when a significant reason was identified for the isolated effect of the factors analyzed or for interaction between them.

Associations between groups and categorical variables were verified by Fisher's exact test. Cohen's effect size (ES) was estimated to outline the differences in the means of the groups with unequal sample sizes within a pre-post-control design and interpreted as follows: 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) e; d ≥ 4.00 (almost perfect effect), according to Hopkins et al.[27] For the purposes of calculating ES, the BFR group was considered as a control group. For the interpretation of d Cohen, the probability of superiority, measured in percentage was used.[28,29]

To verify the relationships between osteocalcin plasma levels and BMD (CL, CF, FT) following the 24 weeks of intervention, Spearman's correlation test (Rho - ρ) was used, according to the following classification: ρ = 0 – 0.01: very low; ρ = 0.1 – 0.3: low; ρ = 0.3 – 0.5: moderate; ρ = 0.5 – 0.7: high; ρ = 0.7 – 0.9: very high; ρ = 0.9 – 1.0: almost perfect,[27] with a significance level of 5%, for all comparisons.

Results

The participants´ sociodemographic information and baseline of lumbar spine, femoral neck and total femur score for osteoporosis classification are presented in Table 1. There were no significant differences in BMD or osteocalcin (p > 0.05) between the groups prior to the training intervention.

As outlined on Table 2, there were no significant group versus time interactions and no significant group or time main effects for the BMD measured at the femoral neck, total femur and lumbar spine regions.

Table 1 - Sociodemographic information about the participants and baseline of lumbar spine, femoral neck and total femur score for osteoporosis classification

	W+BFR (n = 10)	**W** (n = 9)	**BFR** (n = 11)
Age (years)*	64.10 ± 2.50	65.20 ± 5.30	68.40 ± 4.80
Ethnicity n (%)			
Caucasian	5 (50.0)	2 (22.2)	2 (18.2)
African Brazilian	5 (50.0)	2 (22.2)	3 (27.3)
Brown	0 (0.0)	5 (55.6)	6 (54.5)
Body mass (kg)*	64.70 ± 10.50	69.40 ± 8.20	64.90 ± 12.80
Height (m)*	1.52 ± 0.04	1.53 ± 0.05	1.50 ± 0.03
Body mass index (kg/m²)*	28.00 ± 4.10	29.70 ± 3.30	28.60 ± 5.70
Osteoporosis classification			
Lumbar spine score n (%)			
Normal	1 (10.0)	2 (22.2)	2 (18.2)
Osteopenia	6 (60.0)	5 (55.6)	5 (45.5)
Osteoporosis	3 (30.0)	2 (22.2)	4 (36.4)
Femoral neck score n (%)			
Normal	2 (20.0)	2 (22.2)	2 (18.2)
Osteopenia	8 (80.0)	7 (77.8)	7 (63.6)
Osteoporosis	0 (0.0)	0 (0.0)	2 (18.2)
Total femur score n (%)			
Normal	4 (40.0)	6 (66.7)	4 (36.4)
Osteopenia	5 (50.0)	3 (33.3)	6 (54.5)
Osteoporosis	1 (10.0)	0 (0.0)	1 (9.1)

Note: W+BRF = walking with blood flow restriction group; W = walking group; BRF = blood flow restriction group. *Data are presented as mean ± standard deviation.

Table 2 - Absolute bone mineral density values across groups before and after 24 weeks post-training

Region	W+ BFR		W		BFR		p-value		
	Initial	Final	Initial	Final	Initial	Final	G	T	GxT
Femoral neck	0.94 (0.04)	0.95 (0.04)	1.01 (0.05)	1.00 (0.05)	0.94 (0.03)	0.95 (0.02)	0.31	0.93	0.99
Total femur	0.79 (0.03)	0.78 (0.03)	0.89 (0.05)	0.88 (0.05)	0.81 (0.04)	0.80 (0.05)	0.17	0.94	0.99
Lumbar spine	0.84 (0.04)	0.86 (0.04)	0.94 (0.04)	0.93 (0.04)	0.86 (0.05)	0.85 (0.05)	0.06	0.98	0.99

Note: W+BRF = walking with blood flow restriction group; W = walking group; BRF = blood flow restriction group; G = group; T = time; G x T = group versus time interaction. Data expressed as mean (standard error).

As can be seen in Figure 3, there were no differences between groups for osteocalcin concentration (W(2) = 0.27; p = 0.87) following the training intervention. However, it was possible to observe that regardless of the group, all individuals presented values significantly greater 24 weeks post-intervention when compared to the pre-assessment (p = 0.002) and 12 weeks post-intervention (p = 0.006). Thus, regardless of the type of training, positive effects on osteocalcin were observed after 12 and 24 weeks of intervention (W (2) = 10.95; p < 0.01).

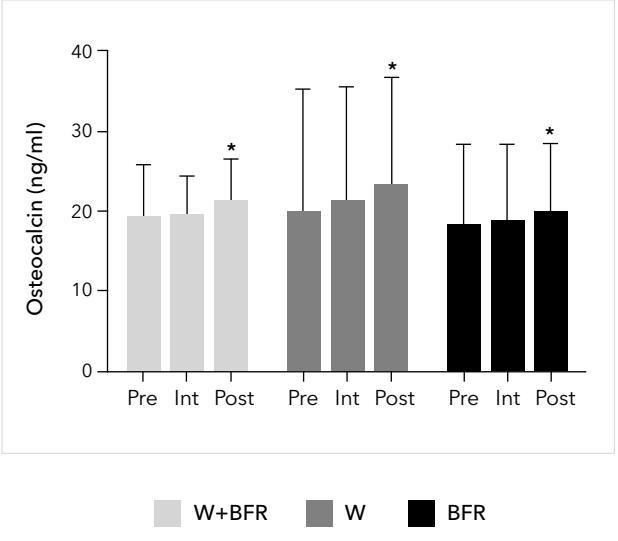

Figure 3 - Osteocalcin concentrations.

Note: W+BFR = walking with blood flow restriction group; W = walk group; BFR = blood flow restriction group; Pre = initial evaluation; Int = evaluation with 12 weeks; Post = evaluation with 24 weeks. *Different from the pre-assessment and the intermediate evaluation.

Trivial effects were observed for the osteocalcin concentration in the W+BFR and W groups when compared to the BFR group, with an effect size of d = 0.027 and d = 0.079 after 12 weeks of intervention and a trivial effect for both experimental groups (W+BFR: d = 0.065; W: d = 0.136) 24 weeks post-intervention. The correlations (Spearman) between BMD (LS, FN and TF) variables versus osteocalcin were not significant.

Discussion

To our knowledge this was the first study that performed a 24 week aerobic training intervention in combination with the BFR in older women with osteopenia/osteoporosis. The main findings of the present study were: 1) there were no differences between groups in BMD of LS, FN and TF; 2) there were no differences between groups in osteocalcin levels; and 3) osteocalcin levels increased at week 12 of the intervention and was accentuated following the 24 week intervention for each protocol (W+BFR, W and BFR). There was a moderate effect, following 24 weeks of training for the W+BFR group.

Hatori et al.[30] reported significant increases in BMD following 28 weeks walking in a similar subject cohort as that in the present study. However, the walking intensity was higher than that in our study, being above the anaerobic threhsold.

Martyn-St James and Carroll[19] observed in their meta-analysis that there was a low, but significant effect of walking, when performed daily, on BMD of the femoral neck (0.014 g/cm^2) but none in the lumbar spine, suggesting that other forms of exercise, which provide greater load, may be necessary to preserve or increase BMD in women after menopause.

In the present study, although there were no significant gains, there was a maintenance of bone mass at the three sites (LS, FN and TF) in every intervention group after 24 weeks. This is notable since the W+BFR and BFR groups used low loads (40% VO_2 peak) and maintained bone mass to the same extent as the W group, which trained with moderate intensity (60% VO_2 peak). The maintenance of BMD in the present study could be due to the increased activation of hypoxia-induced transcriptional factor signaling pathway and the simultaneous activation of vascular endothelial growth factor (VEGF), which induces angiogenesis, consequently, the supply of oxygen and nutrients for osteogenesis.[31] Although not addressed in the present study, it has been shown that in this subject cohort the low BMD is associated with low muscle mass.[32] Hence, it is likely that the exercise protocols herein have also contributed to save muscle mass loss. It is also likely that an increase in interstitial fluid within the long bones could be another mechanism to stimulate bone formation with the BFR technique, by increased venous pressures with localized ischemia.[33]

Although no study has reported improvements in BMD following chronic aerobic training with BFR, one study by Beekley et al.[16] combined walking with BFR and found significant increases in a bone formation biomarker (bone-specific alkaline phosphatase - BAP), though in healthy young volunteers. Indeed, Bittar et al.,[15] in a systematic review of the significance of BFR exercise and bone health, found four studies that used the BAP as a biomarker of bone formation.[16,25,34,35] However, in the present study we opted to use osteocalcin. BAP is specific for bone formation but does not eliminate the cross reaction with the hepatic isoform (15-20%), being more specific and indicative than total alkaline phosphatase, but less specific than osteocalcin.[36]

To satisfy the control condition warranted by randomized clinical studies, we adjusted the effect sizes so that the probability of a participant randomly selected from the W+BFR and W training groups having a plasma osteocalcin level higher than a control group participant (BFR) was 50%. After 24 weeks of intervention, the W+BFR and W groups had a 50% and 52.82% probability, respectively. Beekley et al.,[16] who aerobically trained healthy men aged 21 to 28 years for three weeks obtained a 61.14% probability with the Kaatsu training group to have a plasma BAP level higher than that of an individual in the group control. The magnitude of the effect in relation to the present study can be explained by sex and age differences. Another possible explanation for the small effect sizes in the present study could be the lower blood flow restriction pressure used in the BFR intervention groups, when compared with those in the literature.[16,25] Again, the age of the subjects herein warranted a sharper control of risk with BFR.[22]

Some possible limitations in the present study are the low pressure of blood flow restriction, the short intervention time, the sample size, and the absence of a control group that had not performed any intervention.

Conclusion

The three protocols herein were effective for maintaining BMD and increasing osteocalcin following 24 weeks of the intervention, indicating that BFR is an effective alternative for the preservation of bone mass. Further research is needed with the BFR technique combined with exercise programs longer than 24 weeks in older women and there is a need to study the blood pressure restriction ideal for osteogenesis without any risk to the health of the elderly.

Authors' contribution

STB was responsible for the methodology, research, writing of the original data and, with MSCS, for the project conceptualization and management. STB, HHS and MSCS were responsible for the resources; STB, VMAO, ATSB and RMM, for the data curation; VMAO, RMM and JMVMA, for the formal analysis. STB, HHS and MSCS wrote the article and, along with ATSB and JMVMA, reviewed and edited it. HHS and MSCS supervised all project stages.

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