Effects of blood flow restriction training on bone and muscle tissue: a pilot study

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Summary

The studies completed so far support the hypothesis that low intensity training (LIT) associated with blood flow restriction (BFR) increases muscle hypertrophy (MH) and maximum dynamic force (MDF). However, there is a lack of firm evidence linking this methodology with adaptations in the bone.

The objective of this study was to establish the effect of four LIT protocols associated with BFR, in the MH, MDF, bone mass (BM), bone mineral density (BMD) and bone mineral concentration (BMC) of the lower limb over a period of 11 weeks of training.

Sixteen moderately trained individuals were recruited. A random distribution of the participants was carried out, being distributed. G1: Electro-Neuromuscular Stimulation (ENMS) + BFR; G2: Treadmill walk + BFR; G3: Squat 90º + BFR; G4: Only BFR. Direct measurement of the MDF, Anthropometry and Dual Radiological Densitometry was used to measure the variables. The measurements were made at the beginning and the end of the 11 weeks.

In the MH variable, the walking treatments + BFR and ENMS + BFR registered main improvements compared to the rest of the interventions. The MDF is affected and improved by the ENMS, walking and squats are associated with BFR, in a similar way to the BFR application only. Modifications were observed in BM, BMD and BMC. The ENMS + BFR led the results, improving the BMD and BMC. The walk + BFR showed to improve the BM and the BMD at the same time.

The BFR added to the stimuli, ENMS, walk and squat generates positive effects on the MH, MDF and bone tissue of the lower limb. The BFR also generates changes without the association to another stimulus, but to a lesser extent. It was not possible to achieve a statistically significant difference (p> 0.05) between the groups.

Key words:
Muscle strength. Hypertrophy. Bone. Occlusion. Blood flow.

Efectos del entrenamiento con restricción del flujo sanguíneo sobre el tejido muscular y óseo: un estudio piloto

Resumen

Los estudios completados hasta el momento respaldan la hipótesis de que el entrenamiento de baja intensidad (EBI) asociado con restricción del flujo sanguíneo (RFS) aumenta la hipertrofia muscular (HM) y fuerza dinámica máxima (FDM). Sin embargo, se carece de evidencias firmes que relacionen esta metodología con adaptaciones en el hueso.

El objetivo de este estudio fue establecer el efecto de cuatro protocolos de EBI asociados a RFS, en la HM, FDM, masa ósea (MO), densidad mineral ósea (DMO) y concentración mineral ósea (CMO) del miembro inferior en un periodo de 11 semanas de entrenamiento.

Dieciséis individuos medianamente entrenados fueron reclutados. Se realizó una distribución aleatoria de los participantes quedando distribuidos. G1: Electro Estimulación Neuromuscular (EENM) + RFS; G2: Caminata en treadmill + RFS; G3: Sentadilla 90º + RFS; G4: Solo RFS. Se utilizó medición directa de la FDM, Antropometría y Densitometría Radiológica Dual para medir las variables. Las mediciones fueron realizadas al inicio y al final de las 11 semanas.

En la variable HM los tratamientos de caminata + RFS y EENM + RFS registraron las principales mejoras frente al resto de las intervenciones. La FDM se ve afectada y mejorada por la EENM, la caminata y las sentadillas asociados a RFS, de similar manera a solo la aplicación de RFS. Se observaron modificaciones en la MO, DMO y CMO. La EENM + RFS lidero los resultados, mejorando la DMO y CMO. La caminata + RFS mostro mejorar la MO y la DMO al mismo tiempo.

La RFS sumado a los estímulos, EENM, caminata y sentadilla genera efectos positivos sobre la HM, la FDM y tejido óseo del miembro inferior. La RFS también genera cambios sin la asociación a otro estimulo, pero en menor medida. No se logró establecer una diferencia estadísticamente significativa (p>0,05) entre los grupos.

Palabras clave:
Fuerza muscular. Hipertrofia. Hueso. Oclusión. Flujo sanguíneo.

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Introduction

Low-intensity training (LIT) combined with blood flow restriction (BFR) has been shown to increase muscle strength (MS) and hypertrophy in a similar way to conventional high-intensity resistance exercise. The studies completed to date support the hypothesis that BFR training may provide not only a novel approach to induce adaptation in muscle but also in bone, which was previously thought to occur only with high-intensity and impact exercises. In general, it is necessary to lift loads of approximately 70% of an individual's one repetition maximum (1RM) to obtain significant increases in size and MS, and it is also necessary to perform high-impact activities to stimulate the quality and production of bone tissue (BT)[1-6].

Among the various training instruments and methods to promote MS and muscle hypertrophy (MH), this novel approach has emerged, known as occlusion training (OT), *Kaatsu* Training, Blood flow-restricted exercise (BFRE) or blood flow restriction training (BFRT). It consists in placing an inflatable occlusion cuff or other type of rigid or elastic band around a limb in order to partially reduce the amount of blood delivered to the tissues for a given amount of time, producing hypoxia. This is similar to exercising in anaerobic conditions, with the subsequent physiological and metabolic responses. BFRE is put forward as a unique option for rehabilitation and training, given that it has the potential to produce positive adaptations by training with an intensity that is very similar to that of activities of daily living (10-30% of the maximum workload), improving MS, MH3 and possibly BT[4,7-9].

Low-intensity BFRE offers a unique training option for the development of MH, given that it permits training with an intensity of 20% 1RM and achieves improvements equivalent to those obtained at 65% 1RM, as well as having positive implications for a wide variety of population, specifically senior citizens, the rehabilitation of athletes, bed-bound patients, fractures, cardiac rehabilitation and even astronauts, who are not physically capable of withstanding high mechanical loads[10-15]. Furthermore, there are many beneficial proposals and mechanisms with regard to MS, and a relatively unexplored area relating to BT response.

This study aims to determine whether four low-intensity training protocols combined with BFR are able to produce effects and differences between each protocol, with regard to maximum dynamic strength (MDS), bone mass (BM), bone mineral density (BMD) and bone mineral content (BMC) of the lower limb (LL).

Material and method

A quasi-experimental pilot study was conducted with the convenience selection of 16 participants, forming 4 training groups with 4 participants per group (2 females and 2 males). The participants are moderately trained volunteer students from the first and second years of the Physical Education degree course at the Universidad Católica del Maule (UCM) University, Chile. The subjects completed 11 weeks of training ($\bar{x}$22 ± 4 sessions). With the exception of their classes, they did no extra training during the experimental phase and, therefore, all the physical exercises performed were identical, as detailed in Table 1[16].

Table 1. Characteristics of the sample and distribution of groups.

Groups (G)	$\bar{X}$ - SD	Age (Years)	Weight (Kg)	Height (Cm)	BMI
G1	$\bar{X}$	18.0	62.3	160.25	24.15
NMES + BFR	SD	0.50	8.40	3.75	2.15
G2	$\bar{X}$	19	63.8	164.9	23.3
TW + BFR	SD	1.00	9.90	6.10	1.90
G3	$\bar{X}$	19.33	63.93	164.53	23.53
SQ90º + BFR	SD	0.47	8.03	10.15	0.48
G4 + BFR	$\bar{X}$	19.50	72.00	171.85	24.35
	SD	0.50	5.00	2.15	1.05
Total	$\bar{X}$	19.11	65.33	165.29	23.80
	SD	0.74	8.81	7.88	1.52

BFR: Blood flow restriction; NMES: Neuromuscular electrical stimulation; TW: Treadmill walking; 90ºSQ: 90º squat; BMI: Body mass index; $\bar{X}$: Mean; SD: Standard deviation.

All participants were informed of the aims and duration of the study, giving their written consent to voluntary participate in the same. The research project was approved by the Scientific Ethics Committee of the UCM, under the ethical guidelines laid down in the World Medical Association's Helsinki Declaration on ethical principles for medical research involving human subjects. The dietary variable was controlled by applying the food intake questionnaire (OQ)[17], observing no body-image or eating disorders.

The inclusion criteria were as follows: Be students in academic years 2014 - 2015 of the UCM Physical Education degree course, aged between 17-20 years, male or female gender. The exclusion criteria were as follows: Be a member of a sports team or take part in some type of scheduled and systematic training, be under treatment for a lower limb injury or disability, exhibit a painful lower limb injury or disability. Inclusion in the final analysis required 70% attendance of the sessions.

Techniques and instruments

The measurements were taken in week 1 prior to the intervention and subsequently in week 12, immediately after the training period had concluded. In order to measure the MDS based on 1RM, the direct protocol was used on an incline press set at 90º and adapted to perform it on each limb separately. 90º knee flexion was controlled by an acrylic universal goniometer (Carci brand) with a scale of 0º-360º. The validity, reliability and objectivity levels for the evaluation of maximum strength are based on the recommendations of Brown, (2003) and the ASEP (American Society of Exercise Physiologists)[18].

The "International Working Group of Kinanthropometry" standardised protocol was used for the evaluation of the anthropometric variables[19]. All measurements were taken by a single observer with extensive experience and certified to ISAK level III. Body mass was measured barefoot and wearing as little clothing as possible, using a digital scale with an accuracy of 200 g and a range of 0 to 150 kg (Tanita brand).

Height was measured by positioning the subject, without shoes, in the Frankfurt plane, using an aluminium stadiometer graduated in millimetres and with a scale of 0-2.50 m (Seca brand). Skinfold was measured using a skinfold calliper (Harpenden) which exercises a constant pressure of 10 g/mm. For the circumferences, a nylon tape measure graduated in millimetres was used (Seca brand) with an accuracy of 0.1 cm. The anthropometric body measurements were taken and adapted from the studies made by Vieitez, (2001); Medina, *et al.* (2013) and Norton, *et al.* (1996)[19-21], showing the internationally used validity, reliability and objectivity levels.

For the measurement of the BM, BMD, BMC, bone densitometry (BD) also known as DXA (Dual-energy X-ray Absorptiometry) was used with the protocol proposed by the manufacturer (Lunar Prodigy; General Electric: Fairfield, CT, USA)[22-24]. The accuracy of the DXA is high, with an error margin of 2-6% for body composition[25]. The objectivity of the BM is very high (95-99%),the validity is 85-97%[23].

Procedures

All the training activities and the measurement of variables were conducted at the UCM, specifically at the human performance laboratory located in the technology building, from 15:00 - 17:00 hours. For 3 months, the subjects performed three training sessions a week, on Tuesdays, Thursdays and Fridays, corresponding to the duration of the training, including 10 minutes preparation time, the placement of devices, pre- and post-stretching of the muscle region trained.

The blood pressure cuffs used had a width of 5 cm and a length of 70 cm, with an initial pressure of 110 mmHg. The cuff was adjusted at a pressure of 0 mmHg at the time of placement, measurement and seal. The BFR was applied to the proximal thigh (fifth proximal thigh, measured from the base of the patella to the inguinal crease) applied to the right thigh, remaining in the same position during all the sets as well as the rests.

The preparation and use of the cuffs were based on the review made by Reina, *et al.* (2014), while the initial pressure and the variation parameters were established according to the recommendations made by Loenneke, *et al*. (2014)[14,26].

Task distribution and assignment

Participants were randomly divided into uniform groups, based on gender and group size.

Group 1 (G1): Neuromuscular electrical stimulation (NMES) + BFR 15 minutes of NMES was applied using a consecutive asymmetric two-phase square wave with a pulse width of 300 µs and a frequency of 50 Hz, a current of 40-50 mA, a medium-high perception (it was the maximum tolerated level by subjects) generating a visible muscle contraction. The EMS system (CDM TENS/EMS® Everyway Medical Instruments CO., Ltd. Edition: V1.0) was programmed with a contraction/rest time of 16/0 s. respectively, an 8 s. ramp, 16 s. at ON and 0 s.at OFF[27]. The initial pressure of the cuff was 110 mmHg. Variation during exercise was 110 - 130 mmHg during performance.

4 one-size 4x4 cm adhesive electrode pads were used. These were placed on the motor points of the Quadriceps femoris muscle, respectively under the rectus femoris insertion, 10 cm under each anterosuperior iliac spine, the most prominent area of the vastus medialis and the vastus lateralis. The NMES + BFR was performed with the subject in a seated position, maintaining the feet on the floor with the knee at a 90º angle in each session[27].

Group 2 (G2): Exercise with treadmill walking (TW) + BFR. The subjects performed 5 sets of 2 minutes walking with a 2 minute rest, at a speed calculated according to the Cooper test average speed and controlled with a pulsometer, staying between 50-60% of the maximum heart rate. The HP Cosmos, Mercury model treadmill was used. The initial pressure of the cuff was 110 mmHg. The variation during the performance of the exercise was 110 – 220 mmHg.

Group 3 (G3): Dynamic squat exercise at 90º + BFR. 90º dynamic squats were performed, 5 sets, 2 minute execution, 1 minute rest, pulsometer-controlled execution speed, staying between 50-60% of the maximum heart rate. A 40 cm bench was used to control the knee flexion angle, maintaining it between 0º and 90º during the exercise, with the feet resting at hip height. The initial pressure of the cuff was 110 mmHg. The variation during the performance of the exercise was 110 – 220 mmHg.

Group 4 (G4): BFR alone. BFR alone, in a sitting position for 15 minutes, with the feet resting on the floor and the torso against the backrest. The initial pressure of the cuff was 110 mmHg. There was no pressure variation during the application.

Statistical analysis

The SPSS® (Statistical Package for the Social Sciences) software program for Windows, version 20.0, was used. The measure of central tendency (Mean) was calculated and the measure of spread (Standard deviation), in addition to the simple mathematical calculation of the percentage of progress between the initial and final evaluations for all variables. The Shapiro-Wilk test was performed in order to determine the distribution normality of the data, and the Levene test for the homogeneity of variances, both with a confidence level of 95% and significance of 5% ($p>0.05$). Once the normality of the data and the homogeneity of the variances had been established, the paired t-test was applied in order to compare the pre- and post-evaluations of each group and of the subjects as a whole. The one-way analysis of variance or of one factor (ANOVA) was used to analyse whether there the four treatments differed significantly with regard to their means and variances, in addition to including the Tukey *Post Hoc* test for multiple comparisons. The assumed confidence level was 95% and significance of 5% ($p<0.05$) for these latter tests.

Results

Maximum dynamic strength

The study revealed an increase in the strength indices in all groups. Statistical significance was observed for the t-test in the pre- and post-

Table 2. Effects on the maximum dynamic strength of the lower limb.

	Groups							
	NMES + BFR		TW + BFR		90º SQ + BFR		BFR	
MDS RLL (Kg)	↑*	(1)	↑	(2)	↑	(4)	↑*	(3)
MDS LLL (Kg)	↑*	(1)	↑	(3)	↑	(2)	↑	(4)

Increase (↑); Decrease(↓); No effect (↓); (*) Statistical significance P<0.05; Order of response to treatment (1)>(4); MDS: Maximum dynamic strength. RLL: Right lower limb; LLI: Left lower limb; BFR: Blood flow restriction; NMES: Neuromuscular electrical stimulation; TW: Treadmill walking; 90ºSQ: 90º squat.

evaluation for the subjects as a whole (p=0.002), also for group G1 in the right lower limb (RLL) (p=0.037), left lower limb (LLL) (p=0.028) and for group G4 in the RLL (p=0.049). On the other hand, the groups exhibited differences in their percentages of progress and initial data, making it possible to order and compare their response to treatment (Table 2). However, no significant difference was observed between all the groups for ANOVA in RLL difference (p=0.84), LLL (p=0.66) and between groups for *Post Hoc* in RLL (p=0.845), LLL (p=0.664).

Muscle hypertrophy

With regard to MT (kg), Group 4, solely subjected to the application of BFR, improved by 4.15%, while group G2 which performed TW + BFR improved by 3.78%, as shown in Table 3. This variable decreased in the other two groups. No statistical significance was found for the t-test in the pre- and post evaluations for the subjects as a whole and for each group, nor for the the ANOVA and Post Hoc tests (p>0.05) between the groups. A significant difference was solely observed for the TW group in the muscle tissue to bone tissue ratio (p=0.042).

Bone tissue

The effects observed are varied, as shown in Table 4. Statistical significance was found in the t-test for G2 in the lower limb difference (LLD),

Table 3. Effects on anthropometric indicators related to muscle hypertrophy

	Groups							
	NMES + BFR		TW + BFR		90ºSQ + BFR		BFR	
MT(Kg)	↓	(4)	↑	(2)	↓	(3)	↑	(1)
RMAT	↑	(1)	↓	(1)	↓	(3)	↓	(2)
RMBT	↓	(3)	↑*	(1)	↓	(4)	↑	(2)
MRTC (Cm)	↑	(2)	↑	(1)	↓	(3)	↓	(4)
MeRTC (Cm)	↑	(1)	↑	(3)	↑	(2)	↓	(4)
MRC (Cm)	↓	(3)	↓	(4)	↓	(2)	↓	(1)

Increase (↑); Decrease (↓); No effect (↔); (*) Statistical significance P<0.05; Treatment response order (1)>(4). MT. Muscle tissue; Kg. Kilograms; RMAT. Ratio of muscle to adipose tissue; RMBT. Ratio of muscle to bone tissue; MRTC Maximum right thigh circumference; Cm. Centimetres; MeRTC. Medial right thigh circumference; MRC. Maximum right calf.

Table 4. Effects on the bone tissue.

	Groups							
	NMES + BFR		TW + BFR		90ºSQ + BFR		BFR	
BM RLL	↓	(4)	↑	(1)	↑	(2)	↑	(3)
BM LLL	↓	(4)	↑	(1)	↑	(3)	↑	(2)
BM LLDI	↓	(1)	↑*	(3)	↑	(4)	↓	(2)
BMD RFN	↑	(1)	↓	(3)	↑	(2)	↓	(4)
BMD RFD	↑	(1)	↑	(2)	↑	(3)	↓	(4)
BMD TRF	↑	(1)	↑	(2)	↑	(3)	↓	(4)
BMC RFN	↑	(1)	↓	(3)	↓	(2)	↓	(4)
BMC RFD	↑	(1)	↓	(3)	↑	(2)	↓	(4)
BMC TRF	↓	(1)	↓	(3)	↓	(2)	↓	(4)

Increase (↑); Decrease (↓); No effect (↔);(*) Statistical significance P<0.05; Treatment response order (1)>(4). BM: Bone mass. BMD: Bone mass density. RLL: Right lower limb; LLL: Left lower limb; LLD: Lower limb difference; RFN Right femoral neck; RFD; Right femoral diaphysis; TRF: Total right femur; BMC; bone mineral concentration.

in the BM (p=0.049) and for the pre-and post-evaluation of the subjects as a whole in the BMC of the right femoral neck (RFN) (p=0.046) and total right femur (TRF) (p=0.049). There is no significant difference between the groups (ANOVA) and Post Hoc (p>0.05) for BM, BMD and BMC.

Discussion

This discussion is based on the three parameters that represent the variables analysed herein, with emphasis on the interventions that show limited information in the literature.

Maximum dynamic strength

González-Badillo, *et al.* (2005) conducted a study to determine the influence of the training volume on the strength levels of a group of junior weightlifters. Their conclusions point in the same direction as this study, given that a moderate training volume was shown to be more effective in increasing MS than low or high volumes[28].

Takarada, *et al.* (2000) obtained very similar results, with the BFR group showing strength gains of ~18%, while the conventional training group improved by ~22%. As was the case for our investigation, this study also reported that the differences between groups did not reach statistical significance levels[29].

The study made by Karabulut, *et al.* (2010) is extremely relevant, given that it shows an exercise performed in our investigation, namely the leg extension. The study compares the effects of two types of resistance training protocols on MS in older men. The findings suggest that the leg MS improves with the low-load BFRE and the LIT protocol (20% 1-rm) with vascular restriction, which was almost as effective as the high intensity resistance training protocol (80% 1-RM) for increasing MS in older men. Unfortunately, it does not explain what happened in our study, given that the LL to which BFR was not applied had similar strength gains to the limb with BFR[30].

Muscle hypertrophy

Neuromuscular electrical stimulation and blood flow restriction

Natsume, *et al.* (2015) studied the results of low-intensity NMES combined with BFR on muscle size and MS of 8 healthy, untrained volunteers, of a mean age of 26 years, 174 cm and 71 kg. The training dose used was twice per day, 5 days a week, for two weeks (20 sessions), in the morning and afternoon with a 5-hour interval, seated with a fixed knee angle of 75º, for 23 min. They used two-phase rectangular discharges with cycles of 30 Hz and 8 sec. stimulation with a 3 sec. rest interval, recorded at 5-10% of the maximal voluntary contraction. The BFR device was 10.5 cm. (MT-870 Digital Tourniquet; Mizuho, Tokyo, Japan), using a pressure calculated from the thigh circumference, ranging from 140-200 mmHg., not reporting the variation produced by muscle contraction (which the authors establish as a study limitation), they performed 5 min. sets with 1 min. rests. Their findings show that, after 2 weeks of training, the application of BFR+NMES generated hypertrophy by 3.9% and this decreased by 3% after 2 weeks of detraining. Furthermore, there was an increase in the maximal isometric knee extension strength + 14.2% and isokinetic maximal strength of 7.0% to 8.3%, finding no significant changes with the sole application of NMES, comparing the initial and final evaluation. In addition to the two treatments, no significant differences were obtained using ANOVA and t-test. This work showed an important training density, yet it was only conducted over 2 weeks. Our work made the evaluation with a dynamic leg press test, the dose and time frames differ significantly from our study, although they do confirm a difference in the results with G1 of our study, in the improvements in strength, which were 47.34% in the NMES+BFR group and 20% in the BFR alone group[31].

Another limited report on the combination of NMES and BFR was made by Slysz, *et al*. (2017). The intervention was made for 32 minutes, 4 days a week for six weeks. Leg strength increased 32 ± 19 kg in the NMES+BFR group, which differed from the 3 ± 11 kg change in the control group (p=0.03). The isolated NMES and BFR groups showed increases of 16 ± 28 kg and 18 ± 17 kg, respectively, but these did not statistically differ from the control, or from one to another. There were no statistically significant alterations for the muscle mass. Comparing the results with G1 of our study, the strength improvements were 26 Kg in the NMES group and 16.25 kg for the group that only performed BFR. These results are partly similar to the findings herein, with regard to the fact that the difference in thigh circumference was not significant, which was related to the poor response in terms of muscle mass increase, maximal right muscle circumference (t=0.960) and right medial thigh circumference (t=0.122)[32].

Treadmill and blood flow restriction

Abe, *et al.* (2006) examined the acute and chronic effects of walk training with and without BFR on MRI-measured muscle size and MDS and isometric strength, together with blood hormonal parameters. This study reported a far greater increase of strength in the treadmill walking group, 31.7% in the LL with the BFR device and 20.8% in the one without, which could be due to the fact that they completed 6 weeks of training, three times a week, which confirms the conclusions of Loenneke, *et al.* (2012) in their meta-analysis, whereby a greater frequency of sessions and days per week does not produce better results, and the correlation between a greater number of weeks and strength gains. The characteristics of the subjects with regard to age, body composition, training level and training dose were similar to those of our study[33,3].

Squats and blood flow restriction

Abe, *et al*. (2005) investigated the effects on young subjects of 2 weeks of training performed twice daily, 6 days per week with 3 sets of two exercises, squat and leg curl. The experimental group for BFR + Exercise showed strength increases of 17% for squat and 23% for leg curl, also significantly increasing the IGF-1 growth factor (p <0.01). No significant changes in relative strength were observed in the pre- and post-test for both groups. They conclude that hypertrophy and strength gains in the thigh occur after 2 weeks. In our study, the group performing the squats improved its strength by 19.45%. As in the study described, no significant difference was observed in the test (t=0.337), (p>0.05). Our group completed 11 weeks of training but with fewer sessions, a lower frequency per week and only with squats. We observe that these two protocols, although they differ considerably and have the squat exercise in common, improve the strength of the lower limb in a similar manner. In our study, significant changes were observed in MS with and without BFR, while MH did not show this response, this is due to the use of anthropometry and not measurements to determine the CSA as reported by other studies that specifically evaluated this variable[34].

Bone tissue

Sato, *et al.* (2005) investigated the hypothesis that moderate exercise intensities associated with BFR would generate adaptations in the BT similar to the responses of high intensities. They measured the bone alkaline phosphatase (BALP) found on the osteoblasts responsible for bone formation. Their study was conducted on healthy males subjected to a twice daily walk with BFR on the thighs with a 4-hour interval between sessions, for 3 weeks. The findings were of interest, given that they determined significant increases in the MRI-measured muscle CSA (p <0.01), strength (p <0.01) and BALP (p <0.05), the BALP increase for the experimental group was 10.8% and 0.3% for the control group. There was no significant change in IGF-1 for either group. The authors concluded that 3 weeks BFR walk training increases the BALP, a serum marker of bone turnover. These blood markers were not recorded in our investigation, however the data proposed by these authors could explain the BM and BMD increases that give our treadmill group the leading position for the average response in these variables[35].

In their study, Bemben, *et al*. (2007) proposed to determine the acute effect on the blood markers responsible for BT formation, using low intensity training (LIT) combined with BFR, a single LIT group and a control group. They evaluated 30 minutes after 1 set of 30 reps followed by 3 sets of 15 reps with 30 seconds rest between sets at 20% 1-RM. They evaluated the BALP and cross-linked N-telopeptide (NTx) of type I collagen (bone remodelling biomarker) concluding that a single bout of BFR at 20% 1-RM resulted in decreases in the NTx bone resorption marker but had no effect on the BALP bone formation marker[8].

The study made by Karabulut, *et al*. (2011) compared the effects of different resistance training protocols on bone marker concentrations in older men with a mean age of 56.8 years, for a 6 week duration. The serum concentrations of BALP and NTx improved in both resistance training protocols, suggesting increased bone turnover with a balance favouring bone formation. Therefore, despite using a low mechanical load combined with BFR, this is a potentially effective training alternative to traditional high intensity training for enhancing bone health in older men. They also used BD, not finding any changes in the BM, BMD and BMC, associating this with the training duration of just 6 weeks. As the authors did not provide the breakdown made in our study, we are unable to make an appropriate comparison. However, in a similar way to our findings, the difference values were not significant on comparing the groups[9].

Another of the few works available on the effect of BFR on BT is provided by Loenneke, *et al.* (2013), in a study of the case and rehabilitation of an osteochondral fracture of the right knee of a 22-year-old body-builder, with a height of 175 cm and weight of 70 kg. His progress was favourable, due to the application of BFR, however the conclusions obtained are clinical and individual in terms of pain, radiograph review and magnetic resonance imaging, not providing conclusive data on BM, BMD and BMC, and with no control group to compare the results[36].

Although these prior results show an effect on the bone system, there is an evident lack of studies in this respect. The comparison of our results on BT is an inconclusive task, given the limited number of studies published with regard to the adaptations that this training generates on this tissue in particular, and even less so with the combination of NMES and BFR[2-6,13,37,38]. The study limitations include the small sample size, the convenience selection of participants and no comparison between males and females, which must be addressed and controlled in future investigations.

Conclusions

It can be concluded that BFR combined with low-intensity stimuli such as NMES, treadmill and squat, produces positive effects on the MDS, MT and the BT of the LL. BFR also generates changes when used alone without another stimulus, although to a lesser extent. There is a difference in the response of the MT and BT and also in the MDS to different forms of exercise, being unable to establish a statistically significant difference for moderately trained healthy subjects in an 11 week period.

Future studies should be undertaken in order to further examine the underlying mechanisms of the process of MH induced by a hypoxic stimulus, specifically to clarify the reasons why studies show phenomena such as hypertrophy or MDS gains in non-occluded muscles. Particular importance should be given to the development of an investigation line using a control group to establish a dose-response, adaptations in the short and long term, and to the generation of greater evidence in the area of bone tissue adaptation and rehabilitation.

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Conflict of interest

The authors have no conflict of interest at all.

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