

ORIGINAL ARTICLE

Chronic effects of simultaneous electromyostimulation and vibration on leg blood flow in spinal cord injury

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Study design: Randomized two-group parallel.

Objectives: The objective of this study was to analyze the adaptations on the popliteal artery (mean blood velocity (MBV), peak blood velocity (PBV), arterial resting diameter (RD) and blood flow (BF)) induced by 12 weeks of simultaneous application of whole-body vibration and electromyostimulation (WBV+ES) in patients with spinal cord injury (SCI). Secondly, the musculoskeletal effects of this therapy on the gastrocnemius muscle thickness (MT) and femoral neck bone mineral density (BMD) were analyzed.

Setting: Valladolid, Spain.

Methods: Seventeen SCI patients (American Spinal Injury Association (ASIA) A or B) were randomly assigned to the experimental group (EG = 9) or the control group (CG = 8). Each subject was assessed in four different occasions: at baseline, after 6 weeks (Post-6) and 12 weeks of the treatment (Post-12) and 8 weeks after the end of the treatment (Post-20). Subjects in the EG performed 30 10-min sessions of WBV+ES during 12 weeks.

Results: In the EG, RD increased compared with the baseline value at Post-6 (9.5%, $P < 0.01$), Post-12 (19.0%, $P < 0.001$) and Post-20 (16.7%, $P < 0.001$). Similarly, in the EG, BF increased compared with the baseline value and with CG only at Post-12 ((33.9%, $P < 0.01$) and (72.5%, $P < 0.05$), respectively). Similarly, WBV+ES increased the MT of the gastrocnemius. BMD of both hips remained invariable during the study. CG showed no change at any point.

Conclusions: WBV+ES improved popliteal artery BF, RD and MT after 12 weeks in SCI patients. This increase in RD remained above baseline after 8 weeks. The combination of WBV and ES could be considered a promising alternative to reverse the musculoskeletal atrophy and improve peripheral vascular properties in SCI patients.

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INTRODUCTION

Spinal cord injury (SCI) is one of the most devastating clinical conditions, which can lead to loss of independence and currently has limited potential for recovery.¹ The SCI population has a 228% greater mortality rate from cardiovascular diseases than the able-bodied population.^{2,3} Not surprisingly, young people with chronic SCI experience an accelerated development of cardiovascular diseases due to premature arterial aging and loss of physical ability.^{2,4}

Recent evidence suggests that peripheral arterial dysfunction may be a relevant factor for the increased cardiovascular risk⁵ in this population versus the traditional central cardiac mechanisms.⁶ Traditional cardiovascular risk factors (e.g., sex, age, diabetes, blood lipid profile, elevated systolic blood pressure and smoking status⁷) do not seem to be sufficient to explain the augmented cardiovascular risk in SCI patients.⁸ In this sense, there are several structural and functional adverse arterial adaptations after SCI that could deteriorate cardiovascular health. For example, significant modifications have been described in peripheral arteries below the level of the injury almost immediately after SCI.^{9,10} A previous study reported a 30% reduction in the common femoral artery diameter and blood flow after 6 weeks of SCI.⁹ A 50% and 40% reductions in diameter and blood flow have been observed, respectively, in the common femoral artery after

periods of physical inactivity longer than 6 weeks.⁵ In addition, this early vascular dysfunction has been closely related to the decrease in metabolic demand after SCI.¹¹ The only study that related the common femoral artery diameter with leg muscle volume found no difference between chronic SCI (>1 year) patients and healthy subjects.¹² Although absolute peak blood flow reactivity to thigh muscle ischemia was reduced in SCI patients, adjustment of peak blood flow for the reduced muscle volume eliminated the between-group differences. Therefore, arterial dysfunction and muscle atrophy are strongly associated in SCI patients.¹²

These peripheral adverse vascular adaptations appear to be responsible for the development of pressure ulcers due to the significant reduction in lower limb arterial blood flow.¹³ About 95% of SCI patients will suffer from this secondary complication,¹⁴ which is one of the leading causes of rehospitalization in SCI patients. In addition, arterial dysfunction in SCI is considered a risk factor for developing thromboembolism. Because of a 40–81% prevalence thromboembolism is considered the third leading cause of death in these patients.¹⁵ It has been proposed that cardiovascular mortality associated with chronic SCI is potentially preventable through exercise training.³

Electromyostimulation (ES) and whole-body vibration (WBV) are effective methods to stimulate paralyzed skeletal muscles of SCI

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patients. The effective implementation of ES for the prevention of venous stasis and its related complications in patients with circulatory disease^{16,17} has been widely studied. Similarly, an increment in the femoral artery inflow through ES of the calf muscles¹⁸ has been observed in this population. However, despite the relevance of peripheral arterial dysfunction in SCI patients, the number of studies examining arterial adaptations to ES training is very small. In this line, ES training has shown positive vascular effects^{19,20} in SCI patients. In these studies, SCI patients performed dynamic leg extensions against different resistances evoked by ES. After 18 weeks of training, the authors observed an improvement in muscle fatigue, flow-mediated dilation and arterial range (expressed as maximum diameter – minimum diameter). However, artery size and blood flow did not increase, possibly because of the low-volume protocol used (8 min per week). In contrast, 2 weeks of ES cycling training improved arterial diameter and blood flow in SCI patients.²¹ These findings suggest that the exercise mode combined with ES is important to evoke beneficial arterial adaptations in SCI patients.

WBV produces a stretch-shortening action that activates muscle spindles and triggers reflexive muscle contraction.²² To date, only acute cardiovascular effects of WBV have been examined in SCI patients. In the first study, 3–6 min of WBV elicited increases in oxygen consumption in the gastrocnemius muscle, suggesting increased perfusion.²³ In the second study, the vibratory stimulus applied to the feet produced increases in femoral artery blood velocity.²⁴ Our research group has previously demonstrated that the simultaneous application of WBV and ES (WBV+ES) produced a greater acute popliteal artery blood velocity response than the isolated or consecutive application of both stimuli in healthy males²⁵ and SCI patients.²⁶ However, to date, no study has analyzed the arterial adaptations to this novel method. The purpose of this study was to analyze the induced adaptations on the popliteal artery (mean blood velocity (MBV), peak blood velocity (PBV), arterial resting diameter (RD) and blood flow (BF)) in SCI patients after 12 weeks of WBV+ES.

In addition, the effects of WBV+ES on the gastrocnemius muscle thickness (MT) and femoral neck bone mineral density (BMD) were analyzed.

MATERIALS AND METHODS

Subjects

Seventeen patients (12 males and 5 females) volunteered to participate in the study. All the patients had SCI and used wheelchair for their locomotion. All the participants were classified according to the American Spinal Injury Association (ASIA) as A or B level and was considered as the inclusion criteria. Medications were recorded and only antispasticity drugs were allowed during the study. The possibility that a participant started a different pharmacological treatment during the study was considered as the exclusion criteria. Finally, non-attending more than one therapy session was an exclusion criterion. Table 1 summarizes the characteristics of the sample. All the subjects received ten 2-h rehabilitation sessions per month, consisting of standing position (or tilt position), passive movements, low-intensity resistance training or electrotherapy, and physiotherapy treatment. WBV+ES was applied to the patients before their rehabilitation routines. Each participant gave written informed consent before testing. The study was conducted according to the Declaration of Helsinki and was approved by the University Committee on Human Research. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research.

Experimental design

The effects of the simultaneous application of WBV and ES were analyzed in a randomized two-group parallel design. Patients were randomly assigned to the

Table 1 Descriptive characteristics of the study sample

	Gender	Age (years)	Height (m)	Mass (kg)	Years after injury	Level of lesion	ASIA score
1	M	63	1.68	69.4	29	T9	A
2	M	38	1.92	88.8	13	C7	A
3	M	33	1.80	70.3	4	C6	A
4	M	31	1.76	74.3	9	C7	A
5	M	36	1.74	50.2	17	C7	B
6	M	50	1.85	95.9	4	L1	B
7	M	59	1.70	73.8	20	T2	A
8	M	42	1.77	80.0	24	C7	B
9	M	43	1.74	110.0	8	C7	A
10	M	62	1.75	71.0	6	T12	A
11	M	49	1.72	63.1	7	T6	A
12	M	65	1.70	78.0	5	C4	B
13	F	49	1.64	54.6	12	T7	A
14	F	66	1.56	61.2	8	T3	A
15	F	57	1.60	62.4	12	L1	A
16	F	38	1.58	48.0	27	T4	B
17	F	68	1.62	75.2	22	T4	A
	Mean	49.9	1.72	72.1	13.3		
	S.d.	12.5	0.10	15.9	8.3		

Abbreviations: ASIA, American Spinal Injury Association; F, female; M, male.

experimental group (EG = 9) or the control group (CG = 8). Each subject was assessed in four different occasions: at baseline (B), after 6 weeks (Post-6), after 12 weeks (end of the treatment period, Post-12) and 8 weeks after the end of the treatment period (Post-20). Patients in the EG performed 30 10-min sessions of WBV+ES during 12 weeks.²⁵

Procedures

When a patient came to the laboratory, s/he was seated in her/his own wheelchair with the feet anchored with straps to the vibration platform (Galileo Home, Galileo; Novotec, Nettetel, Germany). To facilitate the placement of the feet, a wedge with an inclination of 20° was used to support the vibrating platform (Figure 1). During all treatments, participants wore the same athletic shoes to standardize the damping of the vibration by the footwear.²⁷ The frequency of vibration was set at 10 Hz and the amplitude at 5 mm (peak to peak). The vertical component of acceleration was measured by an accelerometer (VT-6360, Hong Kong, China). The acceleration (peak) was 6.8 g. Feet were placed parallel to each other 38 cm apart (measured from the midlines of the heels).

For the ES, a rectangular, biphasic and symmetric wave with a pulse width of 400 µs was applied (Compex 3; DJO Ibérica, Madrid, Spain). A continuous 8 Hz current was used during all the protocol, resulting in a pulsatile stimulation of the calf. Three 2-mm-thick self-adhesive electrodes were used on each leg: one electrode (10 × 5 cm²) was placed about 2 cm below the popliteal fold, and two electrodes (5 × 5 cm²) were placed as close as possible to the motor point of the gastrocnemius medialis (GM) and gastrocnemius lateralis (GL), respectively (Figure 2). Current intensity was increased until the patient's motor threshold (mean achieved intensity: 49.5 ± 11.2 mA). This intensity, reached in the first treatment session, was increased during each subsequent session to avoid a muscle contraction intensity below the motor threshold because of muscle fatigue caused by ES.²⁸

Measurements

Vascular parameters. Each patient completed all testing sessions at the same time of day to avoid variations in arterial function. When a subject arrived at the laboratory, a resting period of 10 min was provided to normalize blood flow. Popliteal artery was imaged in a longitudinal section with an ultrasound

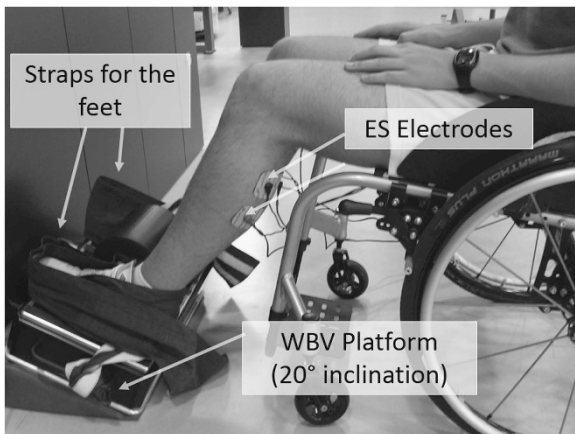


Figure 1 Simultaneous application of WBV+ES.

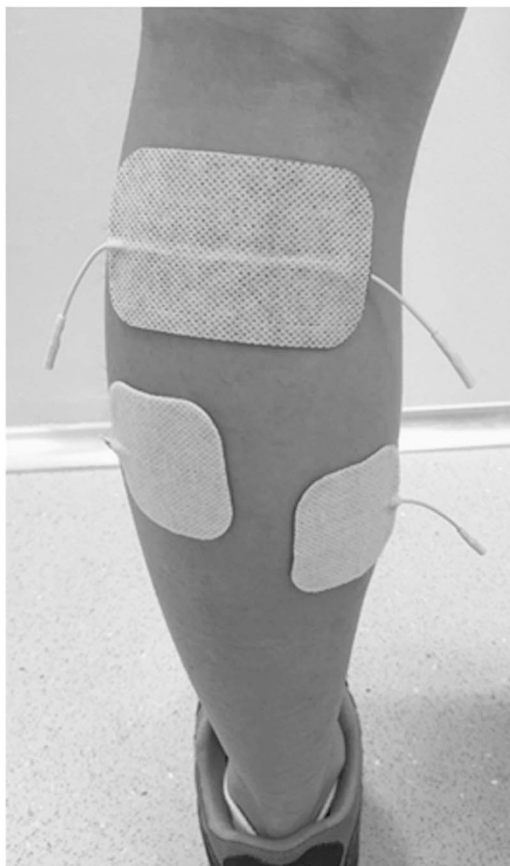


Figure 2 Electromyostimulation electrodes placement.

system (MyLab 25, Esaote Biomedica, Genoa, Italy) using a pulsed color Doppler with a linear array transducer (LA 523, 7.5–12 MHz; length, 50 mm; Esaote Biomedica) placed in the right popliteal fossa. The participant was seated in the wheelchair with a 90° of knee flexion during vascular measurements. The probe was positioned to maintain an insonation angle $\leq 60^\circ$. Each 4 s ultrasound image was recorded by pulsed wave Doppler. These waves were analyzed (MyLabDesk 8.0, Esaote Biomedica) to obtain MBV and PBV. Arterial RD was measured as a perpendicular line from the intima–lumen interface of the near to the far wall. Five images were recorded for each measurement. The highest and the smallest values were excluded, and the mean of the three remaining values was used for further analysis. Images were then analyzed using specialized software (MyLabDesk; Esaote Biomedica). Popliteal artery blood

flow was calculated as $MBV \times \Pi(RD/2)^2 \times 60$. The laboratory personnel were blinded to the randomization. The person who registered and analyzed all the measurements was not the same person who carried out the treatment application. Moreover, an ID number was assigned to each participant during the registration to avoid the identification of the subject during the analysis of the variables studied.

Muscle thickness. MT of GM and GL was assessed using a real-time B-mode ultrasonography linear array ultrasound probe (LA 523, 7.5–12 MHz; length, 50 mm; Esaote). Sagittal plane ultrasound images were obtained at the proximal third between the lateral epicondyle of the femur and the lateral malleolus of the fibula. This distance was measured with the patients lying on an examination bed with their knees fully extended. This site was marked on the skin to ensure the repeatability of the measurement. Subjects were asked to remark this reference everyday during the study period. MT was measured as the distance between superficial and deep aponeurosis. Five images were recorded for each measurement. The highest and the smallest values were excluded and the mean of the three remaining values was used for further analysis. Images were then analyzed using specialized software (MyLabDesk; Esaote Biomedica).

Bone mineral density. The femoral neck BMD was measured using the Norland XR-46 dual energy X-ray absorptiometry (Norland Coopersurgical Corp, WI, USA). Subjects were supine with the foot braced and strapped to a plastic triangular frame, ensuring a hip fixed internal rotation of 60°. BMD was calculated from bone mineral content (g) and bone area (cm²) and expressed as g cm⁻². BMD was assessed at baseline and Post-12.

Data analyses

The normality of the dependent variables was checked and subsequently confirmed using the Shapiro–Wilk test. A two-way repeated-measures analysis of variance in group and time was applied. When a significant F-value was achieved, pairwise comparisons were performed using the Bonferroni *post hoc* procedure. Effect size statistic, η^2 , was provided to determine the magnitude of the effect independently of the sample size. Pearson's correlation coefficient between MT and RD was calculated. Statistical significance was set at $P \leq 0.05$. Values were expressed as mean \pm s.d.

RESULTS

Vascular parameters

Resting MBV, PBV, diameter and BF were not different between EG and CG. MBV and PBV ($P = 0.152$; $\eta^2 = 0.117$ and $P = 0.652$; $\eta^2 = 0.035$, respectively) remained constant along the different time points in both groups (Table 2).

On the contrary, a time \times group effect was observed in RD ($P < 0.001$; $\eta^2 = 0.453$). In the EG, the RD increased compared with baseline value (Figure 3) at Post-6 (9.5%, $P < 0.01$), Post-12 (19.0%, $P < 0.001$) and Post-20 (16.7%, $P < 0.001$). No differences between groups were found.

A time \times group effect was observed in BF ($P < 0.05$; $\eta^2 = 0.202$). In the EG, the BF increased compared with the baseline value (Figure 4) at Post-12 (33.9%, $P < 0.01$). The improved BF after WBV+ES was significant compared with the CG. BF returned to baseline following 8 weeks of detraining.

Muscle thickness

In the left leg (Table 3), a time \times group effect was observed in GL ($P < 0.05$; $\eta^2 = 0.182$) and GM ($P < 0.05$; $\eta^2 = 0.231$). In the EG, MT of the GL increased from baseline to Post-6 (13.5%, $P < 0.001$) and to Post-12 (22.1%, $P < 0.01$). Similarly, MT of the MG increased from baseline to Post-6 (5.8%, $P < 0.01$) and to Post-12 (8.0%, $P < 0.05$). In the right leg (Table 3), a time \times group effect was observed in GL ($P < 0.001$; $\eta^2 = 0.395$) and GM ($P < 0.001$; $\eta^2 = 0.302$). In the EG, MT of the GL increased from baseline to Post-6 (17.5%, $P < 0.001$) and to

Post-12 (18.4%, $P < 0.001$). Similarly, MT of the GM increased from baseline to Post-6 (13.2%, $P < 0.001$) and to Post-12 (17.1%, $P < 0.001$). The increases in LGL (Post-6), LGM (Post-6

and Post-12), RGL (Post-12) and RGM (Post-6) MT following WBV +ES were different compared with the CG. These adaptations were not maintained after the detraining period. Correlations between right gastrocnemius MT and blood flow, as well as right popliteal artery RD, are presented in Table 4.

Table 2 Blood velocity values (expressed in cm s^{-1}) of both groups in the four measurements (mean \pm s.d.)

	EG		CG	
	MBV	PBV	MBV	PBV
Baseline	23.7 \pm 4.3	43.1 \pm 7.7	21.0 \pm 5.9	42.6 \pm 14.7
Post-6	23.9 \pm 6.7	44.0 \pm 10.2	21.6 \pm 4.6	44.0 \pm 13.2
Post-12	22.7 \pm 4.2	40.8 \pm 8.1	18.6 \pm 6.3	39.2 \pm 14.3
Post-20	18.2 \pm 4.6	33.9 \pm 8.7	21.0 \pm 2.8	38.8 \pm 7.1

Abbreviations: CG, control group; EG, experimental group; MBV, mean blood velocity; PBV, peak blood velocity; Post-6 and -12, after 6 and 12 weeks of the treatment; Post-20, 8 weeks after the end of the treatment.

Bone mineral density

The right and left femoral neck BMD remained invariable during the study ($P = 0.176$; $\eta^2 = 0.101$ and $P = 0.555$; $\eta^2 = 0.024$, respectively). For the EG, the right and left (respectively) femoral neck BMD showed no differences between baseline (0.6098 ± 0.1795 and $0.6506 \pm 0.2035 \text{ g cm}^{-2}$) and Post-12 (0.6078 ± 0.1780 and $0.6584 \pm 0.2011 \text{ g cm}^{-2}$). Similarly, for the CG, the right and left (respectively) femoral neck showed no differences between baseline (0.5579 ± 0.1185 and $0.5401 \pm 0.1118 \text{ g cm}^{-2}$) and Post-12 (0.6026 ± 0.1135 and $0.5682 \pm 0.1038 \text{ g cm}^{-2}$).

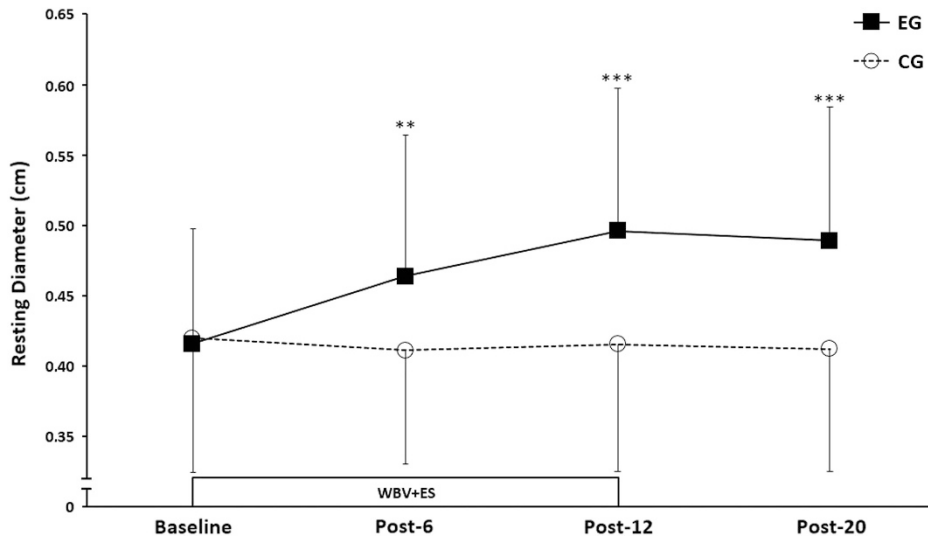


Figure 3 Time-course changes on arterial resting diameter in the four assessments. ** and *** different from baseline at $P < 0.01$ and 0.001 , respectively.

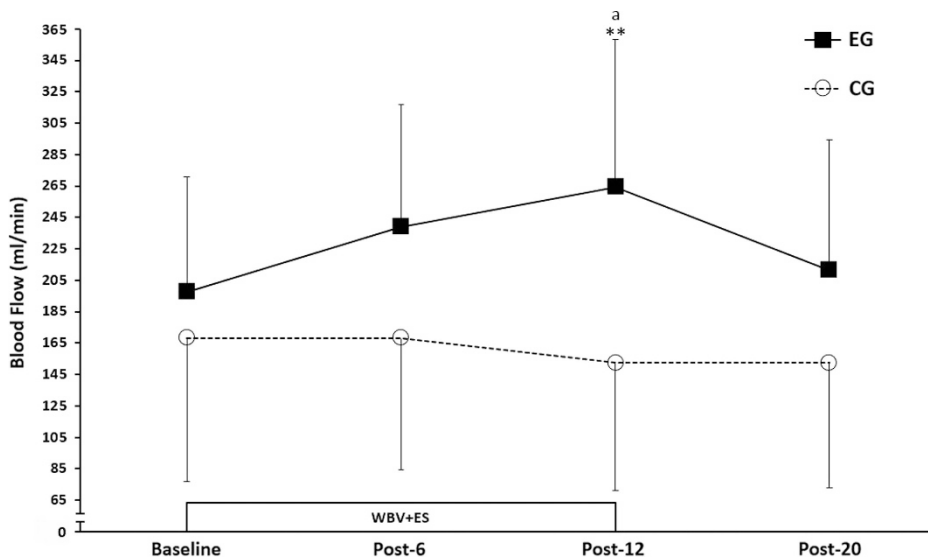


Figure 4 Time-course changes on arterial blood flow in the four assessments. **Different from baseline at $P < 0.01$. ^aDifferent from CG at $P < 0.05$.

Table 3 MT values (expressed in cm) of both groups in the four measurements (mean ± s.d.)

	EG				CG			
	LGL	LGM	RGL	RGM	LGL	LGM	RGL	RGM
Baseline	1.04 ± 0.25	1.38 ± 0.15	1.03 ± 0.23	1.29 ± 0.23	1.00 ± 0.32	1.32 ± 0.20	1.01 ± 0.22	1.29 ± 0.23
Post-6	1.18 ± 0.23 ^{a,***}	1.46 ± 0.13 ^{a,**}	1.21 ± 0.18 ^{a,***}	1.46 ± 0.19 ^{a,***}	0.99 ± 0.24	1.30 ± 0.16	1.02 ± 0.24	1.28 ± 0.26
Post-12	1.27 ± 0.19 ^{**}	1.49 ± 0.16 ^{a,*}	1.22 ± 0.24 ^{a,***}	1.51 ± 0.23 ^{a,***}	1.04 ± 0.24	1.26 ± 0.20	1.01 ± 0.24	1.25 ± 0.27
Post-20	1.13 ± 0.19	1.38 ± 0.14	1.10 ± 0.21 [*]	1.38 ± 0.26	1.03 ± 0.21	1.26 ± 0.18	1.03 ± 0.22	1.22 ± 0.28

Abbreviations: CG, control group; EG, experimental group; LGL, left gastrocnemius lateralis; LGM, left gastrocnemius medialis; Post-6 and -12, after 6 and 12 weeks of the treatment; Post-20, 8 weeks after the end of the treatment; RGL, right gastrocnemius lateralis; RGM, right gastrocnemius medialis.

^{*}, ^{**}, ^{***}Different from baseline at $P < 0.05$, 0.01 and 0.001 , respectively.

^aDifferent from CG at $P < 0.05$.

Table 4 Correlations between right gastrocnemius muscle thickness and blood flow/resting diameter in the four measurements

	BF/RGL	BF/RGM	RD/RGL	RD/RGM
Baseline	0.835 ^{***}	0.841 ^{***}	0.682 ^{**}	0.854 ^{***}
Post-6	0.777 ^{***}	0.834 ^{***}	0.631 ^{***}	0.820 ^{***}
Post-12	0.873 ^{***}	0.860 ^{***}	0.840 ^{***}	0.915 ^{***}
Post-20	0.791 ^{***}	0.820 ^{***}	0.748 ^{***}	0.952 ^{***}

Abbreviations: BF, blood flow; Post-6 and -12, after 6 and 12 weeks of the treatment; Post-20, 8 weeks after the end of the treatment; RD, resting diameter; RGL, right gastrocnemius lateralis; RGM, right gastrocnemius medialis.

^{***}Statistically significant $P < 0.01$ and 0.001 , respectively.

DISCUSSION

This is the first study that has evaluated the chronic application of WBV+ES. Moreover, this is the first time in which SCI patients carried out a training program based on this new therapy. The major finding of this study was that 12 weeks of simultaneous application of WBV+ES enhanced resting BF of the popliteal artery (33.9%) via an increment in the RD (19.0%). This increment of the arterial lumen continued above the baseline value after 8 weeks with no intervention. Despite not being the main outcome of the study, the simultaneous application of both stimuli (WBV+ES) produced an increment in the gastrocnemius MT. However, BMD of both hips remained invariable during the study.

Popliteal artery RD was increased after 6 (9.5%) and 12 (19%) weeks of WBV+ES in SCI patients. In previous studies that examined the chronic effects of high-frequency ES combined with resistance training on vascular properties. The authors reported no influence of electrically stimulated resistance training on femoral artery diameter or blood flow in SCI patients after 18 weeks.^{19,20} In both studies, ES (30 Hz; 450 µs; 5 s ON; 5 s OFF) was applied to perform four sets of 10 dynamic leg extensions during 36 sessions (2 sessions per week). Although these studies applied a tetanic current, a low continuous frequency was used in the present study. It has been documented that these ES currents induce light muscle contractions responsible for a muscle pump effect and therefore evoke acute increases in local muscle blood flow.²⁹ In contrast, tetanic currents with on and off times may lead to partial muscle ischemia and limit the ability to increase blood flow. Accordingly, evoking tetanic contractions could be a limiting factor for structural and functional arterial adaptations following ES-induced exercise training in SCI patients. The reason why we have observed vascular adaptations with respect to these studies could be related with the applied current and its combination with WBV.

Very little is known about the long-term effects of WBV application in the vascular system. To date, no study regarding vascular adaptations after chronic WBV in SCI patients has been carried out. WBV

seems to be responsible for the vascular benefits observed with resistive exercise in healthy subjects confined voluntarily to prolonged bed rest.³⁰ WBV exercise (20–26 Hz) was applied three times per week for 5–7 min per session during 60 days. This protocol attenuated the reduction in diameter of the superficial femoral artery compared with the conventional resistance training and control groups. The vibration stimulus seems to stimulate nitric oxide production and BF in response to increased shear stress,^{30,31} a fundamental mechanism for the prevention of arterial diameter reduction.³² However, Weber *et al.*³³ observed a small increase in the superficial femoral artery RD after 6 weeks of high-intensity resistance training with or without WBV in young healthy men. The ineffectiveness of the superimposed WBV may be explained by a short-term training period and/or lack of skeletal muscle and arterial abnormalities in young men. It is possible that the efficacy of WBV training on arterial function can be apparent in individuals with some degree of dysfunction, as WBV is considered a low-intensity resistance exercise modality.³⁴ Therefore, our study has shown increments in popliteal artery RD and BF after simultaneous therapy with WBV+ES in SCI patients. In previous studies, acute WBV+ES proved to be more effective for increasing popliteal artery MBV and PBV than the isolated application of both treatments in SCI patients²⁶ and healthy young men.²⁵ The hypothetical additional effect of WBV+ES may be due to (i) the recruitment of deeper muscle areas when both stimulus are concomitantly applied and (ii) the larger production of muscle vasodilatory metabolites and endothelial-NO.

The MT of GL and GM of both legs was increased in the EG within the first 6 weeks of the study (Post-6) and to a greater extent in the second half of the treatment period (Post-12). These results are in concordance with previous studies^{35,36} that have shown an increase in muscle size after 8–12 weeks of ES resistance training in SCI patients. Mahoney *et al.*³⁶ used a 'classical' ES resistance training (previously described) two times a week during 12 weeks and observed an increment in the quadriceps femoris cross-sectional area between 35 and 39%. Surprisingly, the simultaneous application of WBV+ES increased MT by 22.1% in the GL in the present study. It should be noted that the remaining muscles analyzed also had a relevant growth. Low-frequency currents have been usually applied as a postexercise recovery modality.²⁹ In this sense, a systematic review concluded that low-frequency ES increases oxidative enzyme activity, although the results concerning changes in muscle fiber composition and muscle size were conflicting.³⁷ However, in a recent case report,³⁸ an ES training protocol consisting of 45 min sessions, 5 days per week for 6 weeks (1350 min total), increases vastus lateralis MT by 3% in healthy men. In this line, we observed an increment of 17.5% in the GL MT at Post-6 with only 150 min of total WBV+ES training. Little is known regarding the neuromuscular and cardiovascular adaptations

produced by this kind of stimulus, and more research is needed on this topic in individuals with disabilities.

On the other hand, WBV has been proposed as a potential method to induce benefits in the musculoskeletal function. Few studies have shown a positive muscular effect by increasing cross-sectional area in postmenopausal women³⁹ or by reducing atrophy induced by prolonged bed rest.⁴⁰ However, these studies involved active voluntary exercise such as squats during WBV. In this sense, the only study that analyzed the chronic application of passive standing on a WBV platform showed no improvements in calf muscles cross-sectional area.⁴¹ Seven SCI adult men (ASIA A or B) completed 40 weeks of passive standing with knees flexed at 160° for 45 min per session with WBV at 45 Hz. Although the WBV was prescribed at a higher intensity and longer periods of acute and chronic exposure than the present study, the intervention did not provide sufficient stimulus to promote muscle hypertrophy in SCI patients. Of clinical interest, our WBV+ES demonstrated relevant results in MT with smaller load training than the previous studies. Therefore, WBV+ES may have greater efficacy than the 'isolated interventions'. It would be interesting to answer this question in the future through a study that carries out a simultaneous isolated comparison in a chronic application. This is a limitation of this study; however, it was not possible to include more groups because of the limited sample available. On the basis of the results of this study, it is not possible to establish what percentage of benefit is due to each treatment. In a previous study,²⁶ this combination induced a higher acute response in popliteal artery blood flow than any isolated treatment. We hypothesized that the chronic effects of applying both treatments concomitantly would be higher than each one alone, resulting in a further impact in the patient's vascular health.

The effect of ES application on BMD has been mainly studied in age- or disuse-induced bone loss in older women and SCI patients. One year of whole-body low-intensity dynamic exercises with ES in older women caused a borderline ($P = 0.051$) increase in lumbar spine BMD but not in hip BMD.⁴² Moreover, the potential benefit of ES for the treatment of osteoporosis resulting from neurological damage is controversial.⁴³ In this new study with recent SCI patients (8 weeks from injury), authors used a high-frequency pattern (30 Hz, 200 μ s) to elicit quadriceps isometric muscle contractions during 5 days a week for 14 weeks. One 47-min session consisted of 10 sets of ES-induced exercise with a 60 s rest between sets. At the end of the study, no effect was observed on hip BMD. Similarly, in our study, no effect in femoral neck BMD was observed after 12 weeks of WBV+ES. First, the location of the scan must be considered, as BMD increments seem to be conditioned to the bone area under mechanical stress.⁴⁴ Second, the intensity of the muscle contraction and the osteogenic effects are closely related.⁴⁵ Therefore, the use of a low-frequency current on the gastrocnemius muscle was insufficient to increase femoral neck BMD in our SCI patients.

Finally, WBV therapy has demonstrated a positive effect on bone remodeling in different special populations such as postmenopausal women.⁴⁶ WBV as a form of resistance training is believed to regulate bone maintenance and stimulate bone formation as shown by increased hip BMD after 6 months.⁴⁷ Despite the characteristic loss of bone mass observed in SCI patients, to date, this aspect has not been widely studied. In a case study with an incomplete SCI patient (4 years from injury), three progressive phases of 10 weeks were applied (standing only, partial standing and combined stand with vibration).⁴⁸ After 20 min of partial standing, the patient was seated in her own wheelchair with her feet on the platform (similar to our study). The participant was asked to perform three isometric exercises during the vibration set (30–50 Hz). In the last phase, the WBV was

applied with the subject standing on the platform. This was the only phase that recorded significant positive changes in BMD at the trunk and spine. With respect to the present study, the position of the participant on the platform created a greater mechanical load.⁴⁸ This is pivotal in the design of specific bone-preservation treatments and could be the main explanation for the absence of BMD effects in our study.

In conclusion, the simultaneous application of WBV+ES produced an increase in popliteal artery BF after 12 weeks in SCI patients. This novel therapeutic method resulted in increased popliteal artery RD after the end of the treatment period. Moreover, this increase remained above baseline after 8 weeks of detraining. The gastrocnemius MT was also increased at Post-12, showing a promising efficiency in SCI patients. These findings could be of great clinical interest to reverse the musculoskeletal deterioration and peripheral arterial dysfunction in SCI patients. These aspects are essential to improve the quality of life and cardiovascular risk in SCI patients. However, this therapy requires further investigation to explore the mechanisms involved in these beneficial adaptations.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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- Jacobs PL, Nash MS. Exercise recommendations for individuals with spinal cord injury. *Sports Med* 2004; **34**: 727–751.
- Phillips WT, Kiratli BJ, Sarkarati M, Weraarchakul G, Myers J, Franklin BA *et al*. Effect of spinal cord injury on the heart and cardiovascular fitness. *Curr Probl Cardiol* 1998; **23**: 641–716.
- Garshick E, Kelley A, Cohen SA, Garrison A, Tun CG, Gagnon D *et al*. A prospective assessment of mortality in chronic spinal cord injury. *Spinal Cord* 2005; **43**: 408–416.
- Hopman MT, Monroe M, Dueck C, Phillips WT, Skinner JS. Blood redistribution and circulatory responses to submaximal arm exercise in persons with spinal cord injury. *Scand J Rehabil Med* 1998; **30**: 167–174.
- West CR, Alyahya A, Laher I, Krassioukov A. Peripheral vascular function in spinal cord injury: a systematic review. *Spinal Cord* 2013; **51**: 10–19.
- Krenz NR, Meakin SO, Krassioukov AV, Weaver LC. Neutralizing intraspinal nerve growth factor blocks autonomic dysreflexia caused by spinal cord injury. *J Neurosci* 1999; **19**: 7405–7414.
- D'Agostino RB Sr, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM *et al*. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation* 2008; **117**: 743–753.
- Krum H, Howes LG, Brown DJ, Ungar G, Moore P, McNeil JJ *et al*. Risk factors for cardiovascular disease in chronic spinal cord injury patients. *Paraplegia* 1992; **30**: 381–388.
- De Groot PC, Van Kuppevelt DH, Pons C, Snoek G, Van Der Woude LH, Hopman MT. Time course of arterial vascular adaptations to inactivity and paralyzes in humans. *Med Sci Sports Exerc* 2003; **35**: 1977–1985.
- Houtman S, Oeseburg B, Hopman MT. Blood volume and hemoglobin after spinal cord injury. *Am J Phys Med Rehabil* 2000; **79**: 260–265.
- Krum H, Howes LG, Brown DJ, Louis WJ. Blood pressure variability in tetraplegic patients with autonomic hyperreflexia. *Paraplegia* 1989; **27**: 284–288.
- Olive JL, Dudley GA, McCully KK. Vascular remodeling after spinal cord injury. *Med Sci Sports Exerc* 2003; **35**: 901–907.
- Cruse JM, Lewis RE, Dilioglou S, Roe DL, Wallace WF, Chen RS. Review of immune function, healing of pressure ulcers, and nutritional status in patients with spinal cord injury. *J Spinal Cord Med* 2000; **23**: 129–135.
- Krause JS, Saunders LL. Health, secondary conditions, and life expectancy after spinal cord injury. *Arch Phys Med Rehabil* 2011; **92**: 1770–1775.
- Prevention of thromboembolism in spinal cord injury. Consortium for spinal cord medicine. *J Spinal Cord Med* 1997; **20**: 259–283.

- 16 Corley GJ, Breen PP, Birlea SI, Serrador JM, Grace PA, O'Leighin G. Hemodynamic effects of habituation to a week-long program of neuromuscular electrical stimulation. *Med Eng Phys* 2012; **34**: 459–465.
- 17 Griffin M, Nicolaides AN, Bond D, Geroulakos G, Kalodiki E. The efficacy of a new stimulation technology to increase venous flow and prevent venous stasis. *Eur J Vasc Endovasc Surg* 2010; **40**: 766–771.
- 18 Abraham P, Mateus V, Bieuzen F, Ouedraogo N, Cisse F, Leftheriotis G. Calf muscle stimulation with the Veinoplus device results in a significant increase in lower limb inflow without generating limb ischemia or pain in patients with peripheral artery disease. *J Vasc Surg* 2013; **57**: 714–719.
- 19 Sabatier MJ, Stoner L, Mahoney ET, Black C, Elder C, Dudley GA *et al*. Electrically stimulated resistance training in SCI individuals increases muscle fatigue resistance but not femoral artery size or blood flow. *Spinal Cord* 2006; **44**: 227–233.
- 20 Stoner L, Sabatier MJ, Mahoney ET, Dudley GA, McCully KK. Electrical stimulation-evoked resistance exercise therapy improves arterial health after chronic spinal cord injury. *Spinal Cord* 2007; **45**: 49–56.
- 21 Thijssen DH, Ellenkamp R, Smits P, Hopman MT, Degens H. Rapid vascular adaptations to training and detraining in persons with spinal cord injury. *Arch Phys Med Rehabil* 2006; **87**: 474–481.
- 22 Rittweger J, Moss AD, Colier W, Stewart C, Degens H. Muscle tissue oxygenation and VEGF in VO-matched vibration and squatting exercise. *Clin Physiol Funct Imag* 2010; **30**: 269–278.
- 23 Yasar-Fisher C, Pascoe DD, Gladden LB, Quindry JC, Hudson J, Sefton J. Acute physiological effects of whole body vibration (WBV) on central hemodynamics, muscle oxygenation and oxygen consumption in individuals with chronic spinal cord injury. *Disabil Rehabil* 2014; **36**: 136–145.
- 24 Herrero AJ, Menendez H, Gil L, Martin J, Martin T, Garcia-Lopez D *et al*. Effects of whole-body vibration on blood flow and neuromuscular activity in spinal cord injury. *Spinal Cord* 2011; **49**: 554–559.
- 25 Menendez H, Martin-Hernandez J, Ferrero C, Figueroa A, Herrero AJ, Marin PJ. Influence of isolated or simultaneous application of electromyostimulation and vibration on leg blood flow. *Eur J Appl Physiol* 2015; **115**: 1747–1755.
- 26 Menéndez H, Ferrero C, Martín-Hernández J, Figueroa A, Marín PJ, Herrero AJ. Acute effects of simultaneous electromyostimulation and vibration on leg blood flow in spinal cord injury. *Spinal Cord* 2016; **54**: 383–389.
- 27 Marin PJ, Bunker D, Rhea MR, Ayllon FN. Neuromuscular activity during whole-body vibration of different amplitudes and footwear conditions: implications for prescription of vibratory stimulation. *J Strength Condit Res* 2009; **23**: 2311–2316.
- 28 Maffiuletti NA. Physiological and methodological considerations for the use of neuromuscular electrical stimulation. *Eur J Appl Physiol* 2010; **110**: 223–234.
- 29 Babault N, Cometti C, Maffiuletti NA, Deley G. Does electrical stimulation enhance post-exercise performance recovery? *Eur J Appl Physiol* 2011; **111**: 2501–2507.
- 30 van Duijnhoven NT, Thijssen DH, Green DJ, Felsenberg D, Belavy DL, Hopman MT. Resistive exercise versus resistive vibration exercise to counteract vascular adaptations to bed rest. *J Appl Physiol* 2010; **108**: 28–33.
- 31 Maloney-Hinds C, Petrofsky JS, Zimmerman G. The effect of 30 Hz vs 50 Hz passive vibration and duration of vibration on skin blood flow in the arm. *Med Sci Monit* 2008; **14**: CR112–CR116.
- 32 Langille BL, O'Donnell F. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science* 1986; **231**: 405–407.
- 33 Weber T, Beijer A, Rosenberger A, Mulder E, Yang P, Schonau E *et al*. Vascular adaptations induced by 6 weeks WBV resistance exercise training. *Clin Physiol Funct Imag* 2013; **33**: 92–100.
- 34 Tapp LR, Signorile JF. Efficacy of WBV as a modality for inducing changes in body composition, aerobic fitness, and muscular strength: a pilot study. *Clin Interv Aging* 2014; **9**: 63–72.
- 35 Dudley GA, Castro MJ, Rogers S, Apple DF Jr. A simple means of increasing muscle size after spinal cord injury: a pilot study. *Eur J Appl Physiol Occup Physiol* 1999; **80**: 394–396.
- 36 Mahoney ET, Bickel CS, Elder C, Black C, Slade JM, Apple D Jr *et al*. Changes in skeletal muscle size and glucose tolerance with electrically stimulated resistance training in subjects with chronic spinal cord injury. *Arch Phys Med Rehabil* 2005; **86**: 1502–1504.
- 37 Sillen MJ, Franssen FM, Gosker HR, Wouters EF, Spruijt MA. Metabolic and structural changes in lower-limb skeletal muscle following neuromuscular electrical stimulation: a systematic review. *PLoS ONE* 2013; **8**: e69391.
- 38 Deley G, Babault N. Could low-frequency electromyostimulation training be an effective alternative to endurance training? An overview in one adult. *J Sports Sci Med* 2014; **13**: 444–450.
- 39 Machado A, Garcia-Lopez D, Gonzalez-Gallego J, Garatachea N. Whole-body vibration training increases muscle strength and mass in older women: a randomized-controlled trial. *Scand J Med Sci Sports* 2010; **20**: 200–207.
- 40 Belavy DL, Miokovic T, Armbrecht G, Rittweger J, Felsenberg D. Resistive vibration exercise reduces lower limb muscle atrophy during 56-day bed-rest. *J Musculoskel Neuron Interact* 2009; **9**: 225–235.
- 41 Masani K, Alizadeh-Meghrizi M, Sayenko DG, Zariffa J, Moore C, Giangregorio L *et al*. Muscle activity, cross-sectional area, and density following passive standing and whole body vibration: a case series. *J Spinal Cord Med* 2014; **37**: 575–581.
- 42 von Stengel S, Bebenek M, Engelke K, Kemmler W. Whole-body electromyostimulation to fight osteopenia in elderly females: The Randomized Controlled Training and Electrostimulation Trial (TEST-III). *J Osteoporosis* 2015, **2015**: 643520.
- 43 Arija-Blazquez A, Ceruelo-Abajo S, Diaz-Merino MS, Godino-Duran JA, Martinez-Dhier L, Martin JL *et al*. Effects of electromyostimulation on muscle and bone in men with acute traumatic spinal cord injury: a randomized clinical trial. *J Spinal Cord Med* 2014; **37**: 299–309.
- 44 Belanger M, Stein RB, Wheeler GD, Gordon T, Leduc B. Electrical stimulation: can it increase muscle strength and reverse osteopenia in spinal cord injured individuals? *Arch Phys Med Rehabil* 2000; **81**: 1090–1098.
- 45 Bloomfield SA, Mysiw WJ, Jackson RD. Bone mass and endocrine adaptations to training in spinal cord injured individuals. *Bone* 1996; **19**: 61–68.
- 46 Weber-Rajek M, Mieszkowski J, Niespodzinski B, Ciechanowska K. Whole-body vibration exercise in postmenopausal osteoporosis. *Menopause Rev* 2015; **14**: 41–47.
- 47 Verschuere SM, Roelants M, Delecluse C, Swinnen S, Vanderschueren D, Boonen S. Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in postmenopausal women: a randomized controlled pilot study. *J Bone Miner Res* 2004; **19**: 352–359.
- 48 Davis R, Sanborn C, Nichols D, Bazett-Jones DM, Dugan EL. The effects of whole body vibration on bone mineral density for a person with a spinal cord injury: a case study. *Adapt Phys Activ Q* 2010; **27**: 60–72.