

# Effects of 4 weeks of low-load unilateral resistance training, with and without blood flow restriction, on strength, thickness, V wave, and H reflex of the soleus muscle in men

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Received: 16 September 2016 / Accepted: 24 April 2017 / Published online: 27 April 2017  
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## Abstract

**Purpose** To test the effects of 4 weeks of unilateral low-load resistance training (LLRT), with and without blood flow restriction (BFR), on maximal voluntary contraction (MVC), muscle thickness, volitional wave (V wave), and Hoffmann reflex (H reflex) of the soleus muscle.

**Methods** Twenty-two males were randomly distributed into three groups: a control group (CTR;  $n = 8$ ); a low-load blood flow restriction resistance training group (BFR-LLRT;  $n = 7$ ), who were an inflatable cuff to occlude blood flow; and a low-load resistance training group without blood flow restriction (LLRT;  $n = 7$ ). The training consisted of four sets of unilateral isometric LLRT (25% of MVC) three times a week over 4 weeks.

**Results** MVC increased 33% ( $P < 0.001$ ) and 22% ( $P < 0.01$ ) in the trained leg of both BFR-LLRT and LLRT groups, respectively. The soleus thickness increased 9.5% ( $P < 0.001$ ) and 6.5% ( $P < 0.01$ ) in the trained leg of both BFR-LLRT and LLRT groups, respectively. However, neither MVC nor thickness changed in either of the legs tested in the CTR group (MVC  $-1$  and  $-5\%$ , and muscle thickness 1.9 and 1.2%, for the control and trained leg, respectively). Moreover, V wave and H reflex did not change significantly in all the groups studied ( $V_{\text{wave}}/M_{\text{wave}}$  ratio  $-7.9$  and  $-2.6\%$ , and  $H_{\text{max}}/M_{\text{max}}$  ratio  $-3.8$  and  $-4\%$ , for the control and trained leg, respectively).

**Conclusions** Collectively, the present data suggest that in spite of the changes occurring in soleus strength and thickness, 4 weeks of low-load resistance training, with or without BFR, does not cause any change in neural drive or motoneuronal excitability.

**Keywords** Neural adaptations · Strength training · Blood flow occlusion · Hypertrophy

## Abbreviations

BFR	Blood flow restriction
BFR-LLRT	Low-load blood flow restriction resistance training
CNS	Central nervous system
CSA	Cross-sectional area
CTR	Control group
H reflex	Hoffmann reflex
iEMG	Integrated EMG
LLRT	Low-load resistance training
MVC	Maximal voluntary contraction
RM	Repetition maximum
RM-ANOVA	Repeated measures analysis of variance
SD	Standard deviation
sEMG	Surface electromyography
VA	Voluntary activation
V wave	Volitional wave

Communicated by Anni Vanhatalo.

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## Introduction

Resistance training promotes strength gains due to muscle hypertrophy and neural adaptations. Traditionally, intensities above 70% of repetition maximum (RM) have been recommended to optimize strength and hypertrophy gains (ACSM 2009). Such intensities have been proven to

be effective in increasing motor unit firing rate (Kamen and Knight 2004) and doublet firings (Van Cutsem et al. 1998), in addition to improvements in the neural drive from descending corticospinal pathways (Aagaard et al. 2002; Vila-Cha et al. 2012; Eklom 2010; Fimland et al. 2009). Low-load resistance training (LLRT) in combination with blood flow restriction (BFR) has been shown to increase human skeletal muscle mass and maximal muscle strength to a similar or greater extent than heavy-load resistance training (Takarada et al. 2000, 2002; Loenneke et al. 2011), at least in the short term and in non-athletic populations. Hypertrophy adaptations include increased type I and II myofiber area, proliferation of myogenic stem cells, and increased numbers of myonuclei, which seem to occur as early as 6–8 days after commencing training (Nielsen et al. 2012). Therefore, this makes the occlusive method an alternative to high-intensity resistance training for increasing strength and muscle hypertrophy, being particularly useful in those populations in which higher loads are not recommended (i.e., elderly or post-surgery rehabilitation patients).

Metabolic stress, along with the reduced oxygen pressure caused by BFR, significantly decreases muscular efficiency, leading to a lower tension and higher metabolic cost (Manini and Clark 2009; Moritani et al. 1992; Yasuda et al. 2010). Because of this, during submaximal contractions, fiber recruitment and type II fiber activation are increased to maintain strength and prevent muscle failure (Moritani et al. 1992). However, it remains unknown whether the structural changes [i.e., increased cross-sectional area (CSA)], together with the increased strength that occurs in response to BFR-LLRT, are accompanied by changes in the central nervous system (CNS), such as those evoked through the traditional resistance training (Kamen and Knight 2004; Van Cutsem et al. 1998; Aagaard et al. 2002; Eklom 2010). Although BFR-LLRT seems to be a poor stimulus for neural adaptation because of the low mechanical stress it induces, BFR application has been shown to increase motor unit recruitment (Moritani et al. 1992; Yasuda et al. 2009, 2010). Furthermore, recent findings have shown that early hypertrophy does not contribute to early strength gains after short-term hypertrophic-oriented training, challenging the prior suggestion that structural adaptations are related to strength improvements (Dankel et al. 2016). Therefore, it is possible that strength gains associated with BFR-LLRT are related to neural adaptations.

In contrast, Kubo et al. (2006) and Moore et al. (2004), using the twitch interpolation technique, found no changes in surface electromyography (sEMG) or voluntary activation (VA) after short-term (8–12 weeks) BFR-LLRT (Kubo et al. 2006; Moore et al. 2004). However, although VA has been shown to be a good marker of central fatigue, it is not a reliable measure of neural adaptation, because

muscles often have only small deficits in VA. Therefore, the improvements obtained in VA with training do not have significant relevance despite increased strength (Shield and Zhou 2004). Consequently, other indices such as the volitional wave (V wave) and the Hoffmann reflex (H reflex) could be better indicators of neural adaptations. These neurophysiological indices, obtained via peripheral electrical stimulation of Ia afferents, reflect the magnitude of motoneuronal excitability or presynaptic inhibition, in the case of the H reflex, or the central descending input from higher motor centers to spinal motor neurons in the case of the V wave (Aagaard et al. 2002). It has been shown that both the H reflex and the V wave are modulated in a specific manner depending on the training stimulus. Heavy strength training increases the V-wave amplitude (Aagaard et al. 2002; Eklom 2010), while the observed H-reflex changes depend on what variant is measured. While the H reflex remains unchanged when it is evoked at rest, it is increased when recorded during contraction (Aagaard et al. 2002; Holtermann et al. 2007). On the other hand, endurance training seems to increase the size of the H reflex, while it does not affect the V wave (Vila-Cha et al. 2012).

Therefore, due to the limitations associated with VA as a neural adaptation index, it seems rational to use other techniques to measure neural adaptations occurring in response to LLRT with or without BFR. Thus, we aimed to measure the V wave and the H reflex to determine whether neural drive and motoneuronal excitability could be influenced by 4 weeks of unilateral isometric LLRT with or without BFR. We hypothesized that only BFR-LLRT would alter neural markers, such as the V wave, H reflex, and sEMG, as seen previously after high-load resistance training without BFR (Aagaard et al. 2002; Eklom 2010; Vila-Cha et al. 2012), due to a greater extent of neural recruitment when BFR is applied when compared to an LLRT regime without BFR.

## Materials and methods

### Participants

Twenty-two healthy right footed (Chapman et al. 1987) and recreationally active men (2–3 h a week of recreational sports activities or aerobic training), with no previous experience in strength training and no history of lower limb disorder, participated in the study. Once the pre-test measures were completed, all the subjects were randomly allocated into three groups: control (CTR;  $n = 8$ ) ( $174.6 \pm 3.8$  cm,  $73 \pm 4.6$  kg,  $24.8 \pm 2.9$  years); LLRT with occlusion (BFR-LLRT;  $n = 7$ ) ( $176.2 \pm 4.5$  cm,  $75.4 \pm 5.8$  kg,  $23.8 \pm 2.5$  years); and LLRT without occlusion (LLRT;  $n = 7$ ) ( $177.1 \pm 5.1$  cm,  $76.8 \pm 6.6$  kg,  $21.5 \pm 3$  years). All subjects gave their written informed consent before being

included in the study, which was approved by the University's Institutional Review Board and conducted in accordance with the latest version of the declaration of Helsinki. All participants came to the laboratory 1 week before the start of the experiment for familiarization with peripheral nerve stimulation, blood flow restriction, and MVC recording. They were required to maintain their usual daily habits and not to take nutritional supplements or start new training programs for the duration of the study. In addition, subjects were asked to refrain from consuming drinks containing caffeine or alcohol, and to avoid exercise 48 h before the testing sessions.

### Electromyography

Recordings were obtained from the belly of the soleus (SOL) of both legs, using Ag–AgCl surface electrodes (3 cm inter-electrode distance) attached to the skin with a belly-tendon assembly (Garland and McComas 1990; Aagaard et al. 2002; Hermens et al. 1999). Electrodes were secured with adhesive tape to prevent possible recording artifacts. EMG signals were amplified (500×), bandpass-filtered (10–500 Hz), and sampled at 1500 Hz with a DTS Desktop (Noraxon, Scottsdale, AZ, USA) connected to an analog–digital board CED Micro1401-3 (Cambridge Electronic Design, Cambridge, UK) for further analysis.

### H and M waves

The soleus motor response (M wave) and H reflex were obtained by electrical stimulation applied on the tibial nerve with the cathode (1.5 cm<sup>2</sup>) located in the popliteal fossa and the anode (5 cm<sup>2</sup>) placed just under the patella. The optimal stimulation point in the popliteal fossa was located with a hand-held electrode using various test stimuli to find the area of the greatest recruitment of Ia afferent nerve fibers, at which point the area for the further recruitment curve was set. Rectangular stimuli of 0.5 ms duration were applied with a constant-current stimulator (DS7AH; Digitimer, Welwyn Garden City, UK). The initial intensity of the electric stimulation, which corresponded to the H-reflex threshold, was gradually increased (0.5 mA steps) to obtain a maximum H wave and the intensity was then increased at intervals of 1 mA until a maximum M wave was obtained and further increases in intensity did not produce increments in M-wave amplitude (Aagaard et al. 2002; Simonsen et al. 1995). The whole process was performed on both legs with the subjects relaxed, sitting with the hip, knee, and ankle joints in a position of 90°. To ensure absolute relaxation of the soleus muscle, the absence of any electromyographic activity in the soleus was verified. Peak-to-peak amplitudes were computed offline from the unrectified EMG signals. Then, maximal H

reflexes were normalized to the corresponding maximal M wave ( $H_{\max}/M_{\max}$  ratio).

### MVC recordings

The subjects, seated with hip, knee, and ankle angles of 90°, performed 3–4 maximal voluntary contractions (MVC) of 3 s of duration with 90 s of rest between trials. In each MVC trial, the conditions were carefully adapted to the experimental details proposed by Gandevia (2001) for valid measurement of maximal isometric strength. For these records, a force transducer (Noraxon force sensor model 520-500 lb-DTS, Noraxon, Scottsdale, AZ, USA) was used, anchored with a metal chain around the handle of an isolation soleus machine that prevented movement of the ankle. After the completion of three valid attempts, the arithmetic mean of the two highest contractions was calculated and used as a reference for subsequent contractions in which the V wave was obtained.

### $V_{\text{wave}}$

$V_{\text{wave}}$  is a variant of the H wave, obtained with a supramaximal electrical stimulation during maximal voluntary contractions.  $V_{\text{wave}}$  reflects the magnitude of the neural drive in descending corticospinal pathways (Aagaard et al. 2002). For the  $V_{\text{wave}}$  recordings, the subjects were required to perform five valid MVCs of 3 s duration with 90 s of rest between trials. In each MVC, a supramaximal stimulus (120% of maximal M wave; 1 ms rectangular pulse) was applied to the tibial nerve. Stimulation was automatically applied 2 s after the beginning of the contraction when the force reached the plateau area. Attempts were discarded if the amplitude of the MVC and M wave did not reach at least 90% of previously recorded values of MVC and  $M_{\max}$ , respectively. Then, peak-to-peak amplitudes of the V and M waves were computed offline from the unrectified EMG signals and expressed as a ratio (i.e.,  $V_{\text{wave}}/M_{\text{wave}}$ ). We also computed the integrated EMG (iEMG) amplitude in a time window of 500 ms prior to the electrical stimulation, and it was then normalized to the amplitude of  $M_{\max}$  in a trial-by-trial fashion ( $i\text{EMG}/M_{\max}$ ).

### Ultrasound

For the sonographic study, subjects lay prone on an examining table. Their legs were relaxed and extended with the ankle at the apex of the table at an angle of 90° (Chow et al. 2000). The soleus images were recorded and analyzed using a Sonosite 180plus equipped with a broadband transducer (Sonosite Inc., Bothell, WA; L38, 38 mm, 5–10 MHz transducer). Scanning was carried out in the sagittal plane and two predetermined sites were scanned. Soleus

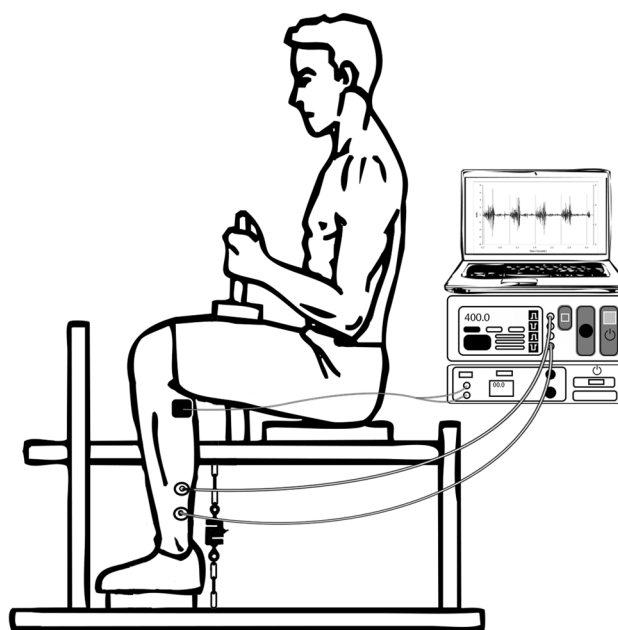
thickness was studied taking positions of 50% (MT50%) and 70% (MT70%) from the proximal part of the distance between the popliteal fossa and the middle part of the lateral malleolus, specifically in the middle area of the lateral half of the leg (Kawakami et al. 1998). Muscle thickness was measured at the center of each scan, in millimeters, as the perpendicular distance between the superficial and deep aponeurosis to which the muscle fiber bundles attached. In the present study, ultrasonic measurement was repeated three times for each subject and averaged values were used. The coefficients of variation of the three measurements were in the range of 0–3%. The reliability for the muscle thickness measurements [ICC = 0.862 (95% CI 0.746–0.928)] was previously determined on two non-consecutive days by the same researcher.

### Training

Both experimental groups, BFR-LLRT and LLRT, performed the same training program. It included only one isometric strength exercise in a specific soleus isolation machine with the hip, knee, and ankle in a 90° angle position (see Fig. 1). The subjects performed four sets with a 30–15–15–15 repetition scheme with an intensity of 25% of the MVC and 30 s rests between sets. All repetitions included 2 s of right leg isometric contraction alternated with 2-s rest periods. This training was carried out 3 days a week, on alternate days for 4 weeks. All subjects completed a total of 12 sessions. The MVC was recalculated weekly for a better training intensity adjustment. The blood flow restriction was applied to the BFR-LLRT group with a 13-cm-wide pneumatic tourniquet for homeostasis (Riester, GmbH, Jungingen, Germany) located in the most proximal part of the thigh (33% of the distance from the inguinal crease to the patella) inflated to a pressure between 150 and 210 mmHg according to the subject's thigh circumference and based on cut points proposed by Loenneke et al. (2013) (45–50 cm = 120 mmHg; 51–55 cm = 150 mmHg; 56–59 cm = 180 mmHg; and 60 cm = 210 mmHg).

### Statistics

The normality and homogeneity of all variables were tested with the Kolmogorov–Smirnov and Levene tests, respectively. Then, three-way repeated measures analysis of variance (RM-ANOVA) was performed, with leg (trained and non-trained), time (pre and post), and group (CTR, BFR-LLRT, and LLRT) as factors for the following variables: MVC, soleus muscle thickness at 50 and 70% of the calf distance, soleus V and M waves, H reflex and  $M_{\max}$ , the  $V_{\text{wave}}/M_{\text{wave}}$  ratio, the  $H_{\max}/M_{\max}$  ratio, and the soleus iEMG/ $M_{\max}$  ratio. When significant interactions of all factors were found, one-way ANOVA on the pre- to post-trial



**Fig. 1** Illustration of the experimental set-up. Hip, knee, and ankle were maintained in a 90° angle position during the training and testing sessions

change scores was used, with Tukey's pairwise post-hoc comparisons. Effect size was calculated using partial Eta squared (partial  $\eta^2$ ) for all the factors of the RM-ANOVA. Cohen's *d* has been used to show the magnitude of the change (effect size) in the paired comparisons. Data are presented as mean  $\pm$  standard deviation in the text and tables, and as mean  $\pm$  standard error in figures. The SPSS 20.0 software (IBM SPSS, Chicago, IL, USA) was used for statistical analysis. Statistical significance was assumed at  $P < 0.05$ .

## Results

### MVC and muscle thickness

Table 1 shows the MVC and muscle thickness values before and after 4 weeks of unilateral isometric LLRT with or without BFR.

The RM-ANOVA for MVC revealed a statistically significant TIME  $\times$  LEG  $\times$  GROUP interaction ( $F(2,19) = 4.61, P < 0.05, \text{partial } \eta^2 = 0.32$ ). The MVC for the trained leg was statistically higher in the post-test than in the pre-test for both the BFR-LLRT ( $P < 0.001, d = 1.88$ ) and the LLRT ( $P < 0.01, d = 0.61$ ) groups, with no changes in the CTR group. With regard to the non-trained leg, a significant improvement in the MVC was found in the BFR-LLRT group only ( $P < 0.01, d = 0.73$ ). Post-hoc analysis showed that MVC changes in both

**Table 1** Mean ± SD values of MVC and thickness measurements before and after 4 weeks of unilateral isometric soleus training

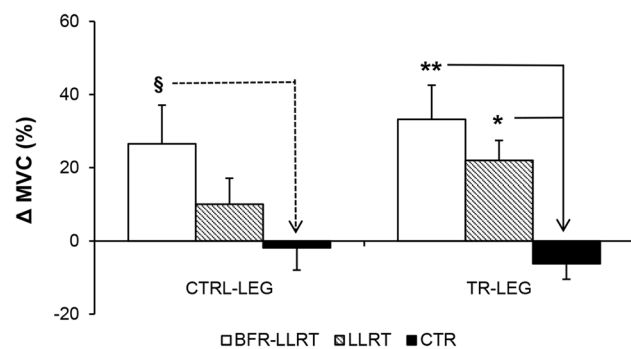
	Control leg		Trained leg	
	Pre	Post	Pre	Post
<b>MVC (N)</b>				
BFR-LLRT	699 ± 220	848 ± 186*	668 ± 124	866 ± 82**
LLRT	765 ± 234	832 ± 233	772 ± 248	931 ± 258*
CTR	760 ± 166	758 ± 232	809 ± 217	768 ± 252
<b>Thickness at 50% (mm)</b>				
BFR-LLRT	15.4 ± 2.1	15.7 ± 2.4	15.8 ± 3.1	17.2 ± 2.9**
LLRT	15.9 ± 1.6	16.1 ± 1.4	15.6 ± 1.6	16.7 ± 1.9*
CTR	15.7 ± 2.5	16.0 ± 2.5	15.6 ± 2.9	15.8 ± 2.8
<b>Thickness at 70% (mm)</b>				
BFR-LLRT	14.8 ± 2.2	15.1 ± 2.4	15.3 ± 2.9	16.5 ± 3.1**
LLRT	14.9 ± 1.8	14.9 ± 1.8	14.6 ± 1.8	15.5 ± 1.8*
CTR	14.7 ± 2.6	14.9 ± 2.6	15.0 ± 2.6	15.2 ± 2.4

CTR control group, BFR-LLRT blood flow restriction low-load resistance training, LLRT low-load resistance training

\*  $P < 0.01$ , \*\*  $P < 0.001$

BFR-LLRT (33%,  $P < 0.01$ ,  $d = 2.05$ ) and LLRT (22%,  $P < 0.05$ ,  $d = 2.08$ ) were significantly higher than the change in the CTR group (−6%, see Fig. 2) for the TR-LEG, and a trend towards significance was found for CTRL-LEG between BFR-LLT and CTR (26.5 vs. −1.8%, respectively;  $P = 0.062$ ,  $d = 1.13$ ).

With regard to SOL thickness, the RM-ANOVA revealed a statistically significant TIME × LEG × GROUP (MT50%:  $F(2,18) = 4.32$ ,  $P < 0.05$ , partial  $\eta^2 = 0.32$ ; MT70%:  $F(1,18) = 4.04$ ,  $P < 0.05$ , partial  $\eta^2 = 0.31$ ) interaction. Soleus muscle thickness was significantly higher in the post-test compared to the pre-test for the trained leg of both experimental groups (MT50%, BFR-LLRT:  $P < 0.001$ ,  $d = 0.46$ , and LLRT:  $P < 0.01$ ,  $d = 0.6$ ;

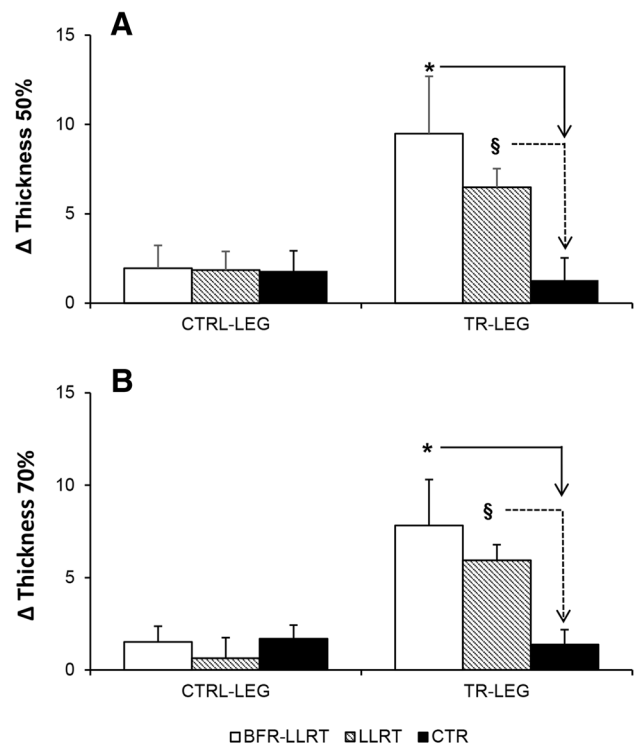


**Fig. 2** Pre- to post-trial group change scores of the MVC in both control and trained legs. CTR control group, BFR-LLRT blood flow restriction low-load resistance training, LLRT low-load resistance training, CTRL-LEG control leg, TR-LEG trained leg. \* $P < 0.01$ , \*\* $P < 0.001$ , §trend towards significance,  $P = 0.062$ ,  $d = 1.13$

MT70%, BFR-LLRT:  $P < 0.001$ ,  $d = 0.39$ , and LLRT:  $P < 0.01$ ,  $d = 0.47$ ; see Table 1), but remained unchanged in the CTR group. The results also showed no significant differences in the soleus thickness for the non-trained leg of any of the three groups tested. The post-hoc analysis showed that the change in thickness of the soleus muscle in the trained leg was significantly higher in the BFR-LLRT group than in the control group (MT50%: 9.5 vs. 1.0%,  $P < 0.05$ ,  $d = 1.24$ , and MT70%: 7.8 vs. 1.0%,  $P < 0.05$ ,  $d = 1.30$ ) and a trend towards a significance between the LLRT and the CTR group (MT50%: 6.5 vs. 1.0%,  $P = 0.16$ ,  $d = 1.83$  and MT70%: 5.9 vs. 1.0%,  $P = 0.13$ ,  $d = 2.08$ ) was also found (see Fig. 3).

**Neural responses**

The results for the neurophysiological parameters are displayed in Table 2. Statistical analysis revealed neither a statistically significant main effect nor an interaction between any of the neurophysiological variables measured (soleus V and M waves, soleus H reflex and  $M_{max}$ , the  $V_{wave}/M_{wave}$



**Fig. 3** Pre- to post-trial group change scores for soleus thickness at 50% (a) and 70% (b) of the distance between the popliteal fossa and the middle part of the lateral malleolus. CTR control group, BFR-LLRT blood flow restriction low-load resistance training, LLRT low-load resistance training, CTRL-LEG control leg, TR-LEG trained leg. \* $P < 0.05$ , §trend towards significance,  $P = 0.16$ ,  $d = 1.83$  in a and  $P = 0.13$ ,  $d = 2.08$  in b

**Table 2** Mean  $\pm$  SD values of  $V_{\text{wave}}$ ,  $M_{\text{wave}}$ ,  $V_{\text{wave}}/M_{\text{wave}}$  ratio,  $H_{\text{max}}$ ,  $M_{\text{max}}$ , and  $H_{\text{max}}/M_{\text{max}}$  ratio measurements before and after 4 weeks of unilateral isometric soleus training

Training	Control leg		Trained leg	
	Pre	Post	Pre	Post
$V_{\text{wave}}$ (mV)				
BFR-LLRT	2.4 $\pm$ 1.1	1.9 $\pm$ 0.8	2.2 $\pm$ 1.0	2.2 $\pm$ 0.8
LLRT	2.2 $\pm$ 1.0	2.0 $\pm$ 0.9	2.3 $\pm$ 1.3	2.6 $\pm$ 1.1
CTR	3.0 $\pm$ 1.6	3.0 $\pm$ 1.5	2.6 $\pm$ 0.8	2.5 $\pm$ 1.2
$M_{\text{wave}}$ (mV)				
BFR-LLRT	6.4 $\pm$ 1.0	6.1 $\pm$ 1.1	6.1 $\pm$ 1.1	6.1 $\pm$ 1.0
LLRT	6.3 $\pm$ 1.5	6.4 $\pm$ 1.7	6.3 $\pm$ 0.8	6.6 $\pm$ 1.0
CTR	6.8 $\pm$ 0.9	6.8 $\pm$ 1.0	6.0 $\pm$ 1.5	6.4 $\pm$ 1.5
$V_{\text{wave}}/M_{\text{wave}}$ ratio				
BFR-LLRT	0.37 $\pm$ 0.14	0.31 $\pm$ 0.11	0.37 $\pm$ 0.17	0.35 $\pm$ 0.09
LLRT	0.35 $\pm$ 0.12	0.31 $\pm$ 0.08	0.36 $\pm$ 0.18	0.39 $\pm$ 0.15
CTR	0.43 $\pm$ 0.19	0.42 $\pm$ 0.19	0.42 $\pm$ 0.13	0.37 $\pm$ 0.16
$H_{\text{max}}$ (mV)				
BFR-LLRT	3.4 $\pm$ 2.1	3.3 $\pm$ 1.4	3.6 $\pm$ 1.8	3.4 $\pm$ 2.1
LLRT	3.5 $\pm$ 1.9	3.2 $\pm$ 1.5	3.1 $\pm$ 1.4	3.3 $\pm$ 1.9
CTR	3.3 $\pm$ 1.1	3.2 $\pm$ 1.6	3.0 $\pm$ 1.7	2.9 $\pm$ 1.8
$M_{\text{max}}$ (mV)				
BFR-LLRT	6.2 $\pm$ 1.8	6.0 $\pm$ 1.5	6.5 $\pm$ 1.5	6.5 $\pm$ 1.0
LLRT	6.5 $\pm$ 1.1	6.7 $\pm$ 2.3	6.3 $\pm$ 1.9	6.5 $\pm$ 2.1
CTR	6.4 $\pm$ 1.1	6.3 $\pm$ 1.4	6.2 $\pm$ 1.6	6.3 $\pm$ 1.3
$H_{\text{max}}/M_{\text{max}}$ ratio				
BFR-LLRT	0.53 $\pm$ 0.22	0.55 $\pm$ 0.18	0.52 $\pm$ 0.18	0.49 $\pm$ 0.25
LLRT	0.55 $\pm$ 0.26	0.48 $\pm$ 0.17	0.49 $\pm$ 0.20	0.49 $\pm$ 0.28
CTR	0.52 $\pm$ 0.22	0.50 $\pm$ 0.19	0.47 $\pm$ 0.20	0.44 $\pm$ 0.22

No significant main effects or interactions have been found in any of the parameters studied

CTR control group, BFR-LLRT blood flow restriction low-load resistance training, LLRT low-load resistance training

ratio, the  $H_{\text{max}}/M_{\text{max}}$  ratio, and the soleus iEMG/ $M_{\text{max}}$  ratio).

## Discussion

This study is the first to investigate the changes occurring in central drive and motoneuronal excitability after LLRT with and without BFR. Higher force capabilities in the soleus muscle (increase in MVC) were found after 4 weeks of isometric LLRT, accompanied by increased soleus thickness.

Several studies have investigated the behaviour of the V and H waves after different types of training stimulus. The evidence indicates that after resistance training, the V wave is increased, while the H reflex measured at rest remains unchanged. (Aagaard et al. 2002; Ekblom

2010; Fimland et al. 2009; Sale et al. 1983; Vila-Cha et al. 2012). However, the opposite happens after endurance training, which directly affects the H-reflex amplitude without changing the V wave (Vila-Cha et al. 2012; Perot et al. 1991). The studies mentioned above show that the early increase in force capability following strength training is partially explained by improvements in neural drive, and can be observed as soon as 3–5 weeks after commencement of training (Ekblom 2010; Vila-Cha et al. 2012). However, all of these studies conducted to address the primary mechanism underlying neural adaptations in response to resistance training have been performed using high-intensity protocols.

Some studies have addressed the problem of neural adaptations after LLRT; however, the techniques used (i.e., sEMG and VA) may not have enough sensitivity to measure possible changes occurring throughout the CNS (Shield and Zhou 2004; Arabadzhev et al. 2014). Consequently, it remains unknown how LLRT can alter the neural structures involved in high-intensity efforts like MVC. Our results clearly showed that 4 weeks (12 sessions) of unilateral isometric LLRT was enough time to increase the soleus MVC and thickness. However, this stimulus did not affect any of the neural markers measured, including the  $V_{\text{wave}}$ , H reflex or the normalized EMG activity recorded during MVC, suggesting a lack of change in motoneuronal excitability and neural drive. This absence of change in V-wave and H-reflex contrasts with our initial hypothesis of enhanced neural adaptations after BFR-LLRT in comparison with LLRT. It is probable that the lack of change in the neural parameters measured in this study is mainly due to a combination of the low intensity used in the training task and the short time period of training. Previous studies have shown increases in V waves after resistance training in a similar short period of 3–5 weeks (Ekblom 2010; Vila-Cha et al. 2012); however, the intensity used in these studies (five sets of five unilateral eccentric repetitions with a load equivalent to a unilateral 1 RM and three sets with an intensity between 60 and 80% of 1 RM, respectively) was much higher than 25% of MVC used in our study. Although BFR has been shown to enhance motor unit recruitment in comparison with the same load without BFR (Moritani et al. 1992), the level of muscle activation seems to be lower than in heavy resistance training (Cook et al. 2013; Manini and Clark 2009) and this could have limited neural adaptations. In addition, although previous studies have shown no changes in MVC performance after repeated MVC testing (Allen et al. 1995; Frost et al. 2012), in our study, both experimental groups performed weekly MVCs, and this could have influenced MVC increments in both experimental groups, possibly reducing the difference in isometric performance adaptations between LLRT and BFR-LLRT due to familiarization with the test. However, weekly MVC

tests along with training stimulus seem not to be sufficient to evoke changes in the measured neural parameters.

The present results are in accordance with previous findings (Kubo et al. 2006; Moore et al. 2004). Moore et al. (2004) found an increase in isometric MVC only after BFR-LLRT in comparison with a non-occluded protocol with the same load; however, their results also revealed no changes in the VA, either in the BFR-LLRT or in the LLRT group after 8 weeks of training. In this regard, Kubo et al. (2006) found that despite the changes observed in the MVC, muscle volume, and physiological CSA after 12 weeks of BFR-LLRT, VA measured using the twitch interpolation technique remained unchanged. However, the same study (Kubo et al. 2006) also revealed that the group required to perform high-load resistance training showed significant increases in both VA and sEMG, together with similar improvements in muscle strength and volume.

However, although all these results seem to support the idea that LLRT, with or without BFR, is not sufficient to evoke neural adaptations, we cannot rule out the possibility that some changes have occurred in the CNS which have not been measured by the techniques used. In fact, the present results revealed a significant increase in the MVC of the control (non-trained) leg of the BFR-LLRT group (21%) without changes in thickness and neural parameters. This increased strength of the untrained limb, known as cross-limb transfer or cross education, is considered to be a consequence of neural adaptations in the corticospinal tract that control this limb (Ruddy and Carson 2013; Lee and Carroll 2007; Carroll et al. 2006; Hortobagyi 2005). Our findings revealed no changes in untrained leg motoneuronal excitability or presynaptic inhibition as measured with the H reflex, which is in accordance with previous cross-transfer studies (Fimland et al. 2009; Lagerquist et al. 2006). However, several studies have suggested that neural adaptations underlying cross education could be located in structures upstream of spinal motoneurons, such as the motor cortex, temporal lobe, or supplementary motor area (Ruddy and Carson 2013; Farthing 2009; Farthing et al. 2007; Lee and Carroll 2007). Furthermore, recent findings have shown that acute sessions of BFR-LLRT produce higher increments in corticospinal excitability, measured with transcranial magnetic stimulation, compared to acute sessions of LLRT (Brandner et al. 2015). It has been argued that this higher excitability in response to a bout of BFR-LLRT is probably due to an altered sensory feedback to cortical or subcortical areas via group III and IV afferents (Brandner et al. 2015). This altered sensory feedback might lead to higher corticospinal and performance adaptations when this type of training is chronically performed. In addition, it has also been shown that latent EMG (activity in the resting limb during unilateral efforts) is increased with fatigue and the intensity of the effort performed by the

trained limb (Aranyi and Rosler 2002; Hopf et al. 1974). Because this bilateral activation during unilateral movements seems to be a consequence of interhemispheric interactions between cortices (Hortobagyi et al. 2003), the higher corticospinal response associated with BFR-LLRT could have increased the mirror activity in the resting limb, leading to an increased cross-transfer effect in comparison with LLRT. Another plausible explanation is that the ischemia produced by the BFR-LLRT enhances the neurophysiological processes of motor learning, due to a decrease in GABA levels within the motor cortex (Levy et al. 2002), making the training more effective and more long-lasting (Cherry-Allen et al. 2015).

Another of the main findings of this study is that both experimental groups showed significant increases in soleus thickness (MT50%: 9.5 and 6.5%; MT70%: 7.8 and 5.9%; in BFR-LLRT and LLRT, respectively) despite the low load used (25% MVC). Although the use of higher loads has been traditionally recommended to promote strength gains and hypertrophy (ACSM 2009), there is increased evidence that low loads, with and without BFR, are as efficient as the higher loads when the volume is enough to transiently create a metabolic stress, which leads to hypertrophic signaling (Barcelos et al. 2015; Burd et al. 2010; Mitchell et al. 2012; Schoenfeld et al. 2014, 2015; Alegre et al. 2015). It has also been shown that BFR may have a higher hypertrophic potential, since metabolic stress is notably higher when occlusion is applied (Pope et al. 2013; Suga et al. 2010, 2012). However, the present results revealed similar hypertrophy adaptations in both LLRT and BFR-LLRT groups. Although it seems contradictory, the present results are in accordance with the most recent literature showing similar adaptations after LLRT with or without BFR (Barcelos et al. 2015; Farup et al. 2015; Wernbom et al. 2013). In this regard, Barcelos et al. (2015) found similar adaptations in CSA, measured with magnetic resonance imaging when different groups of participants performed different training schemes, such as  $1 \times 20\%$ ,  $3 \times 20\%$ ,  $1 \times 50\%$ , and  $3 \times 50\%$  of the RM, independent of the application of BFR. This is also in accordance with studies by Farup et al. (2015) and Wernbom et al. (2013), who found no significant differences in chronic muscle adaptations or in acute hypertrophic signaling. In spite of this, it has been suggested that BFR-LLRT-like muscle plasticity (higher area of type I and type II muscle fibers) is related to a rapid proliferation and differentiation of muscle satellite cells and an increase in numbers of myonuclei, while the earliest hypertrophic processes linked to the LLRT (without occlusion) are more dependent on cell swelling and inflammation processes (Nielsen et al. 2012). We can argue that despite both LLRT and BFR-LLRT groups achieving similar muscle gains, it could be that the hypertrophy of the BFR group was different in nature, which might lead to a higher

muscle growth in the long term (according to the theory of the nuclear domain; Schoenfeld 2010).

In conclusion, the present study is the first empirical evidence showing an absence of adaptations in the Hoffmann reflex and the V wave after 4 weeks of low intensity resistance training either with or without BFR. In addition, results show that increased thickness and isometric strength occur independently of the use of BFR. However, we must be cautious in drawing conclusions because of the short-term nature of the study and the small sample size used. Moreover, further research is needed using different methodologies (e.g., TMS, fMRI, and NIRS) to shed new light on the neural adaptations that occur in response to LLRT.

**Acknowledgements** The authors would like to express their gratitude to the individuals who volunteered to participate in this study. This study was supported by the Spanish Ministry of Economy and Competitiveness (Grant Ref. PSI2015-71061-P).

#### Compliance with ethical standards

**Conflict of interest** The authors declare no actual or potential conflict of interest.

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