Scand J Med Sci Sports 2013: 23: e114–e120
doi: 10.1111/sms.12036

Published by Blackwell Publishing Ltd

SCANDINAVIAN JOURNAL OF
MEDICINE & SCIENCE
IN SPORTS

Muscular adaptations after two different volumes of blood flow-restricted training

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Accepted for publication 23 November 2012

This study aimed to gain an insight into the adaptations of muscle strength and skeletal muscle thickness after two different volumes of blood flow restriction training (BFRT), and compare them with high-intensity training. The sample was divided into four groups: low-volume, low-intensity BFRT (BFRT LV); high-volume, low-intensity BFRT (BFRT HV); traditional high-intensity resistance training (HIT); and a control group, which maintained their routine activities (CON). Leg extension one repetition maximum (1RM), isokinetic peak knee extension, and flexion torques at 60°/s and 180°/s as well as muscle thickness of the rectus femoris (RF) and vastus lateralis (VL) were assessed at baseline and after 5 weeks of training BFRT LV (7.03%, $P < 0.05$), BFRT HV (6.24%, $P < 0.05$) and HIT (18.86%, $P < 0.001$) groups increased 1RM performance, while no changes were observed in the CON group. Muscle thickness of the RF and VL was increased irrespective of the training group (7.5%, $P < 0.001$; and 9.9%, $P < 0.001$, respectively). We conclude that doubling the exercise volume with BFRT causes no further benefit with muscular size or strength. Although similar increases in muscle thickness were observed between training groups, HIT increased 1RM performance to a greater extent compared to either volume of BFRT.

It is assumed that resistance training intensity has to reach levels of at least 60% of one repetition maximum (1RM) to induce significant increases in muscle mass and strength (Campos et al., 2002), while those changes are maximized by training at loads between 80–100% 1RM (Campos et al., 2002; Communications, 2009). However, in the last decade, several articles have shown that training at intensities as low as 20 (Takarada et al., 2004; Abe et al., 2005) –50% (Takarada et al., 2002) 1RM under conditions of restricted blood flow induce increases in muscle strength and stimulate muscle hypertrophy (Loenneke & Pujol, 2009; Abe et al., 2012; Loenneke et al., 2012b).

Since training with blood flow restriction is rather novel, little is known about the most effective protocol to enhance performance. To date, blood flow restriction training (BFRT) protocols have employed training volumes ranging from 45 (Abe et al., 2005) to 75 (Yasuda et al., 2010) repetitions per session. Moreover, it has been demonstrated that 12 weeks of low-load BFRT induce increases in muscle volume and cross-sectional area (CSA) similar to those observed after a traditional resistance training (Kubo et al., 2006).

There is a lack of studies comparing short- or long-term dose-response effects of different blood flow-restricted protocols. Training intensity seems to have a direct influence on acute fatigue. It has been demonstrated that the restriction of blood flow induces muscular fatigue in an inverse relationship with intensity when compared with the same non-occluded exercise (Wernbom & Augustsson, 2006). Additionally, it has been suggested that exercise intensity for muscle hypertrophy should be at least 10% of maximal voluntary contraction in blood flow-restricted muscles (Abe et al., 2012). However, there is no available information concerning the chronic effects of training volume on long-term training adaptations. Understanding the relationships between training volume and adaptations of skeletal muscle thickness and muscular strength is a goal in developing the most effective BFRT protocol. Thus, the purpose of this study was to gain an insight into the adaptations of muscle strength and skeletal muscle thickness after two different volumes of BFRT, and compare them with those induced by traditional high-intensity resistance training.

Materials and methods

Subjects

Thirty-nine recreationally active male university students volunteered for the study. None of the subjects were currently weight training. Exclusion criteria included any known cardiovascular disease or musculo-skeletal problems as this may hinder their ability to resistance train. Prior to data collection, subjects were informed about the risks and benefits of the study and gave their written informed consent. The research project was conducted according to the Declaration of Helsinki and was approved by the University Review Board for use of Human Subjects.

Experimental procedure

During the first week, all subjects came to the laboratory to take part in a familiarization session. After familiarization, subjects were tested before (baseline) and after (post) the 5-week training period. In each testing session, ultrasonographic images were taken to assess muscle thickness; then, after a standardized 10 min warm-up, leg extension 1RM, and isokinetic strength were also assessed. Once baseline values were obtained, subjects were randomly divided into four groups. These groups were named BFRT low volume (BFRT LV, $n = 10$; mean ± SD: age 20.3 ± 1.1 years; height 180.3 ± 4.3 cm; weight 76.9 ± 2.9 kg), BFRT high volume (BFRT HV, $n = 10$; age 21.1 ± 2.0 years; height 177.8 ± 6.6 cm; weight 75.7 ± 7.5 kg), high-intensity training (HIT, $n = 11$; age 20.7 ± 2.3 years; height 180.1 ± 5.8 cm; weight 75.2 ± 10.5 kg), and control (CON, $n = 8$; age 20.2 ± 0.8 years; height 178.4 ± 5.3 cm; weight 75.6 ± 6.4 kg). Subjects in the experimental groups performed bilateral knee extension exercises using an isotonic leg extension machine. Training was performed on two nonconsecutive days per week for 5 weeks. All groups performed a standardized warm-up before training. Warm-up consisted of 5 min of pedaling on a cycle ergometer at 70 W at a cadence of 60–70 rpm. BFRT LV performed 1 set of 30 repetitions followed by 3 sets of 15 repetitions (1 set of 30 + 3 sets of 15; Takano et al., 2005; Fujita et al., 2007; Yasuda et al., 2011). An interset rest interval of 1 min was allowed. BFRT HV doubled the exercise volume of BFRT LV after 5 min rest (1 set of 30 + 3 sets of 15, 5-min rest, 1 set of 30 + 3 sets of 15). Both BFRT groups trained at an intensity of 20% of their previously estimated concentric 1RM. HIT group performed a bodybuilding-type resistance training, consisting of three sets of eight repetitions with 1-min interset rest interval. The intensity of exercise was set at 85% of their previously estimated concentric 1RM. A metronome was used to standardize a lifting cadence of 1.5 s:1.5 s for all groups throughout the full range of motion. Subjects in the control group were asked to maintain their routine activities.

In BFRT groups, a blood flow restriction stimulus was given by compressing the proximal end of both thighs using a pneumatic cuff (RiesterKomprimeter, Riester, Jungingen, Germany). The cuff was 140 mm width and 940 mm length. Immediately before each training session, a pressure of 110 mmHg was applied (Takarada et al., 2000; Patterson & Ferguson, 2010). Pressure remained constant during the training session. In the BFRT HV group, the cuff was removed during the 5-min rest period, whereas in the BFRT LV, pressure was maintained during the whole session. Pressure was released immediately after the end of the last set in both groups.

1RM testing

Maximal isotonic leg extension strength was determined using a bilateral monoarticular leg extension exercise, performed with a leg extension machine (SuperGym, SG8019 Leg Ext/Hamstring Combo, Qingdao Impulse Group Co., Ltd., Shandong, China). Subjects sat on the machine and were instructed to keep their arms crossed over their chest to avoid any synergic movement of the upper body. Subjects' low back was in permanent contact with the back of the machine during the test. The knee moved through a range of 90°, approximately. Subjects began with a load that was estimated to be equivalent to a 50% 1RM and they were instructed to perform eight repetitions. Then, the load was adjusted to the subjects' estimated 75% 1RM and they were instructed to perform five repetitions. These initial two sets served as a specific warm-up. After that, the weight was adjusted and the subject was told to perform repetitions until volitional failure. If they succeeded to complete more than five repetitions, the load was increased by 5%; if they did not, 1RM was estimated by the Epley's formula (see below). Between the first three trials, 3 min of rest were allowed. Further trials required 5 min of rest to ensure recovery. A repetition was considered valid if the subject used proper form and lifted the weight through the entire range of motion. On average, three trials were required to complete the 1RM test.

Epley's formula (Le Suer et al., 1997):

$$1\text{RM} = \text{Load (kg)} \bullet (1 + (0.033 \bullet \text{number of repetitions}))$$

Isokinetic knee extension torque

At least 15 min after the assessment of the leg extension 1RM, concentric isokinetic knee extension and flexion torques of the subjects' dominant leg was measured at 60°/s and 180°/s using an isokinetic dynamometer (Biodex System IV, Biodex Medical Systems Inc., Shirley, New York, USA). Before testing, the input axis of the dynamometer was aligned with the axis of rotation of the knee. The back of the subjects was adjusted to create a hip joint angle of 90°, and a seatbelt was secured across the subjects' thigh, pelvis, and chest. This was done to reduce any movements of the hip and minimize assistance from other muscle groups. Prior to isokinetic testing, the limb weight was gravity corrected by measuring the torque exerted on the dynamometer with the limb positioned at 180° of leg extension (full leg extension) and completely relaxed. During each maximal exertion, subjects were instructed to kick out and pull back as hard and fast as possible. Subjects performed a single set of five repetitions of leg extension and flexion at 60°/s and then, 10 repetitions of leg extension and flexion at 180°/s. Three minutes of rest were allowed between sets. Concentric flexion and extension peak torques at each velocity were recorded for each subject. The hamstring: quadriceps ratio (H:Q ratio) was then calculated as follows:

$$\text{H}:\text{Q} = \frac{\text{Flexion peak torque } (N/n)}{\text{Extension peak torque } (N/n)} \bullet 100$$

Muscle thickness assessment

Muscle thickness was assessed at rest, with subjects performing no physical activity before testing. Muscle thickness was measured by real-time B-mode ultrasonography linear array ultrasound probe (LA 523, 7.5–12 MHz; length of the probe, 50 mm; Esaote Biomedica, Genoa, Italy). After applying hypoallergenic, water-soluble transmission gel, ultrasound transducer was placed on the surface of the skin. Muscle thicknesses of rectus femoris (RF) and vastus lateralis (VL) were assessed. The measurement site was determined at a point midway between the greater trochanter and the lateral epicondyle of the knee. This distance was measured with the subjects 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 experimental period. Transversal images were recorded from the RF; afterwards, the probe was rotated to record longitudinal images from the VL. 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, Genoa, Italy).

Table 1. Baseline and post isokinetic torques (N/m) and effect sizes at 60°/s

		Extension			Flexion			H:Q ratio		
		Baseline	Post	*d*	Baseline	Post	*d*	Baseline	Post	*d*
BFRT LV	Mean	222.4	232.8	0.32	125.2	123.6	–0.07	56.5	53.2	–0.43
	±SD	32.1	30.2		22.2	18.4		7.8	4.6	
BFRT HV	Mean	211.9	217.5	0.18	116.8	125.4	0.46	55.4	58.4	0.43
	±SD	30.3	43.8		18.7	21.5		7.0	9.0	
HIT	Mean	228.1	243.0	0.45	129.7	126.3	–0.15	57.1	52.7*	–0.56
	±SD	33.2	35.7		21.9	20.3		7.8	9.4	
CON	Mean	228.1	232.0	0.13	146.1	143.0	–0.15	64.6	61.8	–0.33
	±SD	32.4	34.8		20.5	23.0		8.5	6.5	

*Significantly different from baseline value ($P < 0.05$).
BFRT LV, low-volume BFRT; BFRT HV, high-volume BFRT; HIT, high-intensity resistance training; CON, control group; *d*, Cohen's *d*.

Table 2. Baseline and post isokinetic torques (N/m) and effect sizes at 180°/s

		Extension			Flexion			H:Q ratio		
		Baseline	Post	*d*	Baseline	Post	*d*	Baseline	Post	*d*
BFRT LV	Mean	154.6	164.2	0.70	103.2	101.9	–0.08	66.6	62.0	–0.56
	±SD	13.7	13.0		17.6	18.3		8.1	9.6	
BFRT HV	Mean	145.5	148.4	0.11	91.4	94.9	0.21	63.5	65.0	0.15
	±SD	26.1	27.0		18.3	12.4		9.6	9.9	
HIT	Mean	160.7	173	0.68	104.4	100.8	–0.23	65.2	58.8	–0.76
	±SD	18.0	24.8		15.5	14.5		8.4	8.1	
CON	Mean	161.1	166.5	0.23	116.3	113.7	–0.16	72.8	68.5	–0.42
	±SD	23.2	20.2		16.7	11.6		10	3.8	

BFRT LV, low-volume BFRT; BFRT HV, high-volume BFRT; HIT, high-intensity resistance training; CON, control group; *d*, Cohen's *d*.

The same researcher took all images. The researcher's intraclass correlation coefficients (ICCs) were 0.998 for the RF muscle thickness ($P < 0.001$) and 0.989 for the VL muscle thickness ($P < 0.001$). Coefficients of variation (CV) were 1.08 and 2.85, respectively. These variations agree with the ICCs and CVs reported in other studies (Csapo et al., 2011).

Statistical analyses

The normality of the data was checked and subsequently confirmed with the Shapiro–Wilk test. A two-way analysis of variance (ANOVA) on group (BFRT LV, BFRT HV, HIT, and CON) and time (baseline and post) was applied. When a significant F-value was detected, pairwise comparisons were performed using the DMS post-hoc procedure. The reliability of ultrasound measurements was assessed with ICC and CV. Effects sizes were measured by partial eta square (η^2) for the ANOVA and also by Cohen's d for comparison between pre- and post-values. Cohen's effect sizes were interpreted as follows: $d < 0.2$ = null effect, $d < 0.5$ = small effect, $d < 0.8$ = medium effect, and $d > 0.8$ = large effects (Cohen, 1988). The level of significance was fixed at an alpha of ≤ 0.05.

Results

Muscle strength

At baseline, all groups showed similar isokinetic leg extension peak torques at 60°/s and 180°/s. Following the training period, a time main effect for increasing leg extension peak torque was detected at 60°/s (3.9%, $P < 0.01$, $\eta^2 = 0.215$) and 180°/s (4.8%, $P < 0.01$, $\eta^2 = 0.285$). No between-group differences were observed post-training for any of the tested leg extension velocities. Additionally, no differences were observed at any point for isokinetic knee flexion. Tables 1 and 2 show isokinetic leg extension and flexion peak torques for all conditions at 60°/s and 180/s, respectively.

While all groups presented similar H:Q ratios at baseline at all velocities, a time main effect for the H:Q ratio was observed at 180°/s (5.1%, $P < 0.01$, $\eta^2 = 0.188$). However, there were no between-group differences in the H:Q ratio observed post-training at180°/s. At 60°/s, the H:Q ratio was lower at post (7.7%, $P < 0.05$) compared to baseline only in the HIT group, while no differences were observed between the BFRT or CON groups.

There were no between-groups differences in 1RM performance at baseline. Following the training period, 1RM performance was increased irrespective of the training group ($P < 0.001$, $\eta^2 = 0.555$) and there was also a time × group ($P < 0.001$, $\eta^2 = 0.434$) interaction. 1RM strength was increased at post in the BFRT LV (7.03%, $d = 0.48$, $P < 0.05$), BFRT HV (6.24%, $d = 0.29$, $P < 0.05$) and HIT (18.86%, $d = 1.19$, $P < 0.001$) groups, while no changes were observed in CON ($d = 0.13$). The post-exercise increase in 1RM strength was greatest for the HIT group compared to the BFRT groups. Figure 1 shows leg extension 1RM values for each time and condition (Table 3).

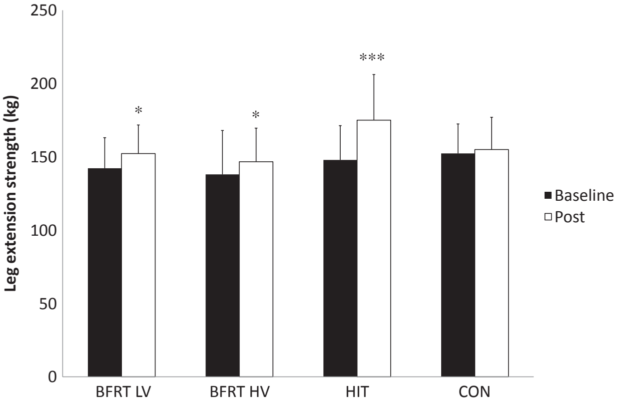

Fig. 1. Baseline and post-leg extension 1RM values for all conditions. *Significantly different from baseline ($P < 0.05$). ***Significantly different from baseline ($P < 0.001$).

Table 3. Baseline and post 1RM values and effect sizes for all conditions

		Baseline	Post	Cohen's *d*
BFRT LV	Mean	142.2	152.2*	0.48
	±SD	20.8	19.5	
BFRT HV	Mean	138	146.7*	0.29
	±SD	29.9	22.9	
HIT	Mean	147.9	175***	1.19
	±SD	23.4	31.1	
CON	Mean	152.3	155	0.13
	±SD	20.1	22	

*Significantly different from baseline value ($P < 0.05$); ***Significantly different from baseline value ($P < 0.001$).

BFRT LV, low-volume BFRT; BFRT HV, high-volume BFRT; HIT, high-intensity resistance training; CON, control group; *d*, Cohen's *d*.

Muscle thickness

RF and VL muscle thicknesses were assessed before and after the training period. There was no between-group difference in muscle thickness observed at baseline or at post for either the RF or VL. After the training period, a time main effect for increasing muscle thickness was observed for both the RF (7.5%, $P < 0.001$, $\eta^2 = 0.475$) and VL (9.9%, $P < 0.001$, $\eta^2 = 0.487$). Figure 2 shows percent changes for RF and VL muscle thicknesses.

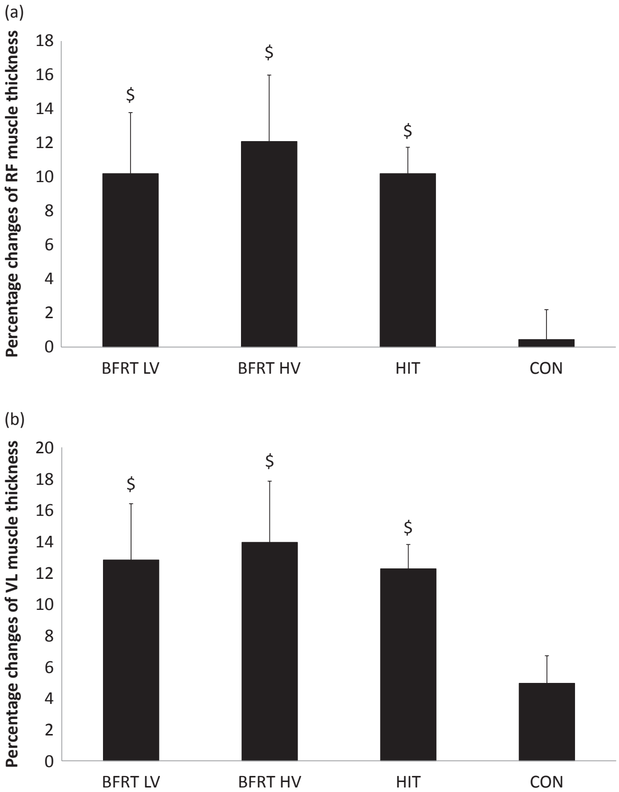

Fig. 2. Percentage changes of RF (a) and VL (b) muscle thickness for all conditions. Values are presented as means + SE. $^{\$}$Significantly different from baseline ($P < 0.05$).

Discussion

To our knowledge, this is the first study comparing the effects of two different volumes of BFRT with the effects induced by traditional resistance training. The main finding of the present study was that there is no relationship between the dose of low-load BFRT and the adaptations of muscular strength and muscle thickness.

The data from the present study indicate that increases in leg extension 1RM strength may be reached after 5 weeks of BFRT independently of the training volume. We also observed that increases in 1RM were greater in the HIT group compared to CON and BFRT groups. In this respect, Cohen's *d* for muscle strength indicates a large effect size for HIT, while a small effect size was observed for both BFRT LV and BFRT HV. These results differ from those observed in other studies in which BFRT has been shown to induce increases in muscle strength to a similar extent as traditional resistance training (Kubo et al., 2006; Karabulut et al., 2010; Clark et al., 2011; Laurentino et al., 2012). It has been stated that following traditional resistance training, increases in strength during the four initial weeks are mainly caused by neural factors rather than structural adaptations (Sale, 1988). It has been suggested that this traditional training adaptation paradigm could be reversed with BFRT, so neural adaptations could occur much later (> 8 weeks) when training with BFRT (Loenneke et al., 2012b). In support of this idea, the recent meta-analysis by Loenneke and colleagues (Loenneke et al. 2012b) revealed a significant correlational relationship between muscle strength and weeks of BFRT, while no significant correlation was found for hypertrophy. In our study, 5 weeks of BFRT could have not been long enough to induce neural adaptations of the quadriceps muscles. In addition, it might be that the 1RM was greater with the higher intensity group because they had more practice with the task of lifting a heavy load on that particular piece of equipment. To illustrate this point, the torque measurements, which were completed on a piece of equipment that no group had "practice" on throughout the duration of the study, produced no group differences between BFRT and HIT.

Isokinetic extensor strength was increased either at 60°/s (3.9%) or 180°/s (4.2%) in all groups. Despite the lack of within-group differences, effect sizes indicate that this increment trended to be higher at 180°/s than at 60°/s. These results agree with those observed in other studies after BFRT, in which increases in isokinetic strength were larger at higher velocities than at low velocities (Takarada et al., 2004; Patterson & Ferguson, 2010). Considering that in those studies BFRT was performed at slow cadences, it seems that increases in isokinetic strength induced by BFRT are not specifically velocity dependent. However, selecting training velocities near to those measured in the isokinetic tests could reveal better testing performances (Sumide et al., 2009).

The blood flow restriction stimulus *per se* may have an effect promoting muscle hypertrophy or, at least, attenuating atrophy of non-exercising muscles (Takarada et al., 2000). The present study was the first to hypothesize that the H:Q ratio could be preserved after the training period in the BFRT groups. In this sense, BFRT HV was the only group in which a small effect size for increasing isokinetic flexion performance at all velocities was observed. Moreover, medium effects for impairment of isokinetic flexion were detected in HIT and BFRT LV at 180°/s. These results support the fact that BFRT HV was the only group that trended to improve the H:Q ratio after training. It has been previously demonstrated that the blood flow restriction stimulus itself may decrease disuse atrophy during a non-weightbearing period (Takarada et al., 2000; Kubota et al., 2008). Additionally, it has been described that after an unloading period, muscular weakness can occur independently of muscle atrophy (Kawakami et al., 2001). Hence, blood flow restriction, even without exercise, is plausible to have had an effect on neuromuscular function of the hamstrings. However, in our study, hamstrings' CSA, muscle thickness, and electromyographic activity were not assessed. Further studies are required to clarify if the blood flow restriction stimulus could have any long-term effect on the non-exercising muscles.

Muscle thickness of RF and VL was increased in a similar extent in all exercising groups after the training period. Exercise groups in the study by Kubo et al. (2006) followed similar training protocols both in the traditional resistance training group and the BFRT group. They found no within-group differences neither in muscle volume and CSA of quadriceps muscles. The same results have been reported in other studies comparing the effect of BFRT and traditional resistance training (Takarada et al., 2000; Laurentino et al., 2012). Thus, it seems that BFRT induces increases in muscle mass in a similar extent as traditional resistance training. Physiological processes underlying BFRT-induced muscle hypertrophy remain unclear. It has been previously demonstrated that the same BFRT LV protocol (1 set of 30 + 3 sets of 15, 20% 1RM) induced an acute relative increase of muscle protein synthesis of 56% (Fry et al., 2010). Moreover, Fujita et al. (2007) registered an increase in protein synthesis along with an activation of the mammalian target of rapamycin signaling pathway 3 h after this protocol. In addition to this, a downregulation of proteolytic markers has also been observed 8 h post-exercise in blood flow-restricted muscles (Manini et al., 2011). Thus, it seems that increases in post-exercise muscle protein synthesis are probably similar between traditional resistance training exercise and BFRT (Abe et al., 2012). Our data, as well as previous evidence, suggests that BFRT has the potential to induce adaptations of muscle thickness similar to those observed after traditional resistance training.

Our overall data indicate that adaptations of muscle mass and strength are not affected by doubling the BFRT volume. Therefore, adaptations to BFRT do not occur in a linear dose-response fashion because of the fact that there appears to be a volume threshold over which further increases in volume are not advantageous (Loenneke et al., 2011). This relationship has been previously observed after traditional resistance training (González-Badillo et al., 2005; Marshall et al., 2011). Nevertheless, there also might be a lower threshold below the BFRT LV that could be enough to induce significant increases in muscle mass and strength. One of the possible limitations of the present study could be the absence of a "very low" BFRT volume group to elucidate whether BFRT LV is the lowest volume threshold to induce adaptations of muscle size and strength or it is not. Future research is needed to address this issue.

In addition, another limitation of the current study is the use of an arbitrary pressure of 110 mmHg for each individual (independent of the limb size) in the BFR resistance training groups. This is important as it has been recently observed for the lower body that leg circumference directly affects the pressure required to restrict blood flow (Loenneke et al., 2012a). Therefore, it is conceivable that 110 mmHg caused greater restriction on some individuals than others. However, there are currently no guidelines for pressures based on thigh circumference, so future studies should be completed to better determine a more uniform pressure.

In summary, BFRT has the potential to increase muscle strength and muscle thickness after a 5-week training period, though the magnitude of these adaptations seems to be independent of the training volume (75 reps vs 150 reps). Additionally, structural adaptations induced by traditional resistance training are similar to those observed following high-volume or low-volume BFRT, while traditional resistance training might induce larger short-term increases in muscle strength as compared to BFRT.

Perspectives

Low-load blood flow-restricted training has shown to induce muscle hypertrophy regardless of training volume. A myriad of protocols, with varying intensities, volumes, and rest intervals have shown to induce muscle hypertrophy under conditions of restricted blood flow. However, understanding the structural adaptations to different volumes of low-intensity BFRT was a goal in developing the most efficient BFRT protocol. Because of the great effort needed to complete a single BFRT session, this training modality has been recommended for use with highly motivated individuals. Therefore, in order to spare time and effort, a maximum of 75 repetitions per session should be recommended per muscle group as the adaptation appears to be maximized at that volume. This may have implications in both training and rehabilitation settings. However, future research is needed to ascertain whether a lower volume of BFRT could drive similar muscular adaptations.

Key words: hypertrophy, dose-response, muscle thickness, KAATSU, occlusion training, strength.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the European Social Fund and Junta de Castilla y Leon, Consejería de Educación through the P.O. Castilla y Leon 2007–2013 program. Authors would also like to thank all participants for their effort.

Conflict of interest: The authors declare no conflict of interest.

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