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Changes in muscle architecture induced by low load blood flow restricted training

J Martín-Hernández[1,2], PJ Marín[1,2], H Menéndez[2], JP Loenneke[3], MJ Coelho-e-Silva[4], D García-López[1], AJ Herrero[1,2]

[1]Faculty of Health Sciences, Miguel de Cervantes European University, Valladolid, Spain
[2]Research Centre on Physical Disability, ASPAYM Castilla y León Foundation, Valladolid, Spain
[3]Department of Health and Exercise Science, University of Oklahoma, Norman, OK, USA
[4]Faculty of Sport Sciences, University of Coimbra, Coimbra, Portugal

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In order to ascertain whether differing structural mechanisms could underlie blood flow restricted training (BFRT) and high intensity training (HIT), this study had two aims: (i) to gain an insight into the acute variations of muscle architecture following a single bout of two different volumes of BFRT, and (ii) to compare these variations with those observed after HIT. Thirty-five young men volunteered for the study and were randomly divided into three groups: BFRT low volume (BFRT LV), BFRT high volume (BFRT HV) and traditional high intensity resistance training (HIT). All subjects performed a bilateral leg extension exercise session with a load of 20% of one repetition maximum (1RM) in the BFRT groups, whereas the load of the HIT group was equivalent to an 85% of their 1RM. Before and immediately after the exercise bout, ultrasound images were taken from the rectus femoris (RF) and the vastus lateralis (VL). All groups increased their RF ($p < 0.001$) and VL ($p < 0.001$) muscle thickness, while the increases in pennation angle were larger in HIT as compared to BFRT LV ($p = 0.013$) and BFRT HV ($p = 0.037$). These results support the hypothesis that acute muscle cell swelling may be involved in the processes underlying BFRT induced muscle hypertrophy. Furthermore, our data indicate differing structural responses to exercise between BFRT and HIT.

Keywords: ultrasound, occlusion training, pennation angle, KAATSU, muscle thickness

The contractile properties of the muscle, such as contraction speed or force generating capacity are influenced by muscle architecture (3, 12). In this sense, long-term traditional high intensity training (HIT) is known to increase muscle pennation angle and cross-sectional area (CSA), while reducing fascicle length. Similarly, a single bout of cycling exercise (4) or repetitive jumps (11) have shown to acutely increase muscle thickness and fascicle's pennation angle. One repetition maximum (1RM) is defined as the maximum amount of weight that one can lift in a single repetition of a given exercise. In this sense, the fact that a mechanical load of at least 70% 1RM should be used to induce increases in CSA and pennation angle following HIT has long been demonstrated (6). However, there is increasing evidence indicating that loads as low as 20% 1RM combined with blood flow restriction may also result in skeletal muscle hypertrophy (19).

Several mechanisms have been proposed to explain the skeletal muscle hypertrophy observed following low intensity blood flow restricted training (BFRT). In this sense, low

Corresponding author: Juan Martín-Hernández, BsC
Faculty of Health Sciences, Miguel de Cervantes European University
C/Padre Julio Chevalier, 2, 47012 Valladolid, Spain
Phone: +34 983 00 1000; Fax: +34 983 278 958; E-mail: jmartinh@uemc.es

intensity BFRT has shown to stimulate muscle protein synthesis 3 h after the exercise (8, 9) and to acutely reduce proteolytic transcriptions (20). Other explanations, such as acute muscle cell swelling have also been proposed (2, 16). Muscle cell hydration state may act both as an anabolic proliferative stimulus when the cell swells and as a catabolic signal when the cell shrinks (10). Cell swelling can be indirectly measured through variations in muscle thickness or muscle volume. In line with this, Fry et al. (8) have reported acute increases in leg circumference after an acute bout of low intensity BFRT. This increment was significantly greater than that observed in the control group, which performed the same exercise without blood flow restriction. Similar results have been obtained after a single bout of BFR walk training (23).

However, to our knowledge, there is no study that has assessed the variations of fascicle arrangement after a single bout of low intensity BFRT. Also, the acute structural response of the muscle may differ between low load BFRT and traditional high intensity resistance training. Understanding the acute structural response to a low intensity BFRT protocol may help us to gain valuable insight into the physiological processes underlying BFRT-induced muscle hypertrophy. Thus, the purpose of this study was twofold, (i) to gain an insight into the acute variations of muscle architecture following a single bout of two different volumes of BFRT, and (ii), to compare these variations with those observed after HIT.

Materials and Methods

Subjects

Thirty-five young, physically active males volunteered for the study. None of them was currently undergoing regular resistance training. All subjects that presented with cardiovascular, metabolic or musculoskeletal diseases that would hinder their ability to perform high intensity resistance training were excluded from the study. All subjects were informed about the risks and benefits that could be derived from their participation in the experiment. Written informed consent was obtained from all subjects prior to participation. The study received approval from the University's Institutional Review Board for the use of Human Subjects and was conducted according to the Declaration of Helsinki.

Experimental procedure

All subjects underwent a familiarization session prior to the experiment. After familiarization, subjects attained at the laboratory on two different occasions, separated by three days at least. During the first session, the leg extension concentric 1RM was obtained for all subjects. Then, the sample was randomly divided into three groups: low intensity (20% 1RM), low volume blood flow restricted training (BFRT LV); low intensity, high volume blood flow restricted training (BFRT HV) and HIT (85% 1RM). Physical characteristics of each group are displayed in Table I. Upon arriving at the laboratory on the second session ultrasound images were obtained at rest from subjects' rectus femoris (RF) and vastus lateralis (VL). A warm-up, consisting of pedaling on a magnetically braked cycle-ergometer (Ergoselect 100, Ergoline, Bitz, Germany) during 5 min with an output power of 70 Watts and a cadence of 60–70 rpm, was performed prior to the exercise session. Then, subjects in the BFRT LV group performed one set of thirty repetitions followed by three sets of fifteen repetitions (9) with a between-sets rest interval of one minute (30 + 3 × 15, 1-min rest). BFRT HV group doubled the exercise volume of BFRT LV with five minutes rest within bouts (30 + 3 × 15 – 5 min – 30 + 3 × 15). The HIT group performed three sets of eight repetitions with inter-set rest intervals of one minute (3 × 8, 1-min rest). Exercise was performed in the same leg extension machine where the 1RM had been previously assessed (SuperGym, SG8019 Leg Ext/Hamstring Combo, Qingdao Impulse Group Co., Ltd. Mainland, China). The lifting cadence was standardized for all groups using a metronome, allowing a cadence of 1.5:1.5 (s) for the concentric and eccentric phase throughout the whole range of motion. A pressure cuff – 140 mm wide, 940 mm length – (Riester Komprimeter, Riester, Jungingen, Germany) was inflated to an arbitrary pressure of 110 mmHg (21, 24, 27) and placed around the proximal end of both thighs with the intent to restrict arterial blood flow into the muscle of interest and occlude venous outflow of that muscle in the BFRT groups. Pressure remained constant during the whole training session and was released immediately upon the completion of the last set. In the BFRT HV, the cuff was released during the 5-min rest between-bouts to allow recovery. Ultrasound images of rectus femoris and vastus lateralis were obtained immediately after the completion of the exercise protocol (post reperfusion in the BFR groups).

Table I. Characteristics of subjects by training group

		Age (yr)	Height (m)	Weight (kg)
BFRT LV	Mean	20.3	1.80	76.9
	± SD	1.1	0.04	2.9
BFRT HV	Mean	21.1	1.78	75.7
	± SD	2.0	0.04	7.5
HIT	Mean	20.7	1.80	75.2
	± SD	2.3	0.04	10.5

Values are means ± SD; BFRT LV ($n = 11$): low volume occlusive training; BFRT HV ($n = 12$): high volume occlusive training; HIT ($n = 12$): high intensity resistance training

Ultrasound measurements

Ultrasound measurements were performed before and after the completion of the exercise protocol. Muscle architecture was measured by real-time B-mode ultrasonographic linear array ultrasound probe (LA 523, 7.5–12 MHz; length of the probe, 50 mm; Esaote Biomedica, Genoa, Italy). None of the subjects reported performing any physical activity prior to testing that could have influenced the ultrasound measurement. Subjects lie supine on an examination bed with their knees fully extended, and rested for 10 min while their skin was shaved and cleaned with alcohol. The measurement site was chosen as the point midway between the greater trochanter and the lateral epicondyle of the knee. This site was marked on the skin with indelible ink to ensure the repeatability of the measurement. Hypoallergenic water-soluble transmission gel was applied on the probe to serve as a conducting interface between the probe and the skin. Then, the probe was placed transversely on the RF and five ultrasound images were obtained. After that, the probe was rotated, tilted and placed longitudinally over the VL until the superficial and deep aponeuroses of the muscle were well defined and parallel to each other. Five longitudinal images were obtained from the VL. Muscle thickness was measured as the perpendicular distance between superficial and deep aponeuroses, whilst pennation angle was measured as the angle between the most visible fascicle and the deep aponeuroses (Fig. 1). Images were analyzed with specialized software (MyLabDesk, Esaote, Genoa, Italy). After the analysis, the highest and the smallest value of each group of five images were excluded. The mean of the remaining three values was used for further analysis. Ultrasound images were acquired before and immediately after the exercise session (post reperfusion in the BFR groups).

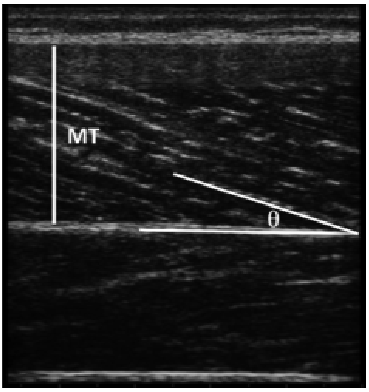

Fig. 1. Muscle thickness (MT) and pennation angle assessment of an ultrasound image of the vastus lateralis (Θ)

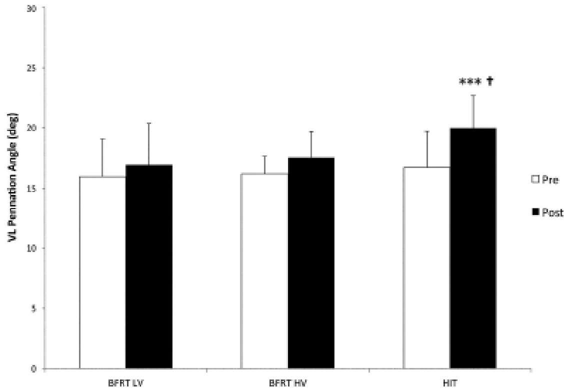

Fig. 2. Pre-post variations of vastus lateralis (VL) pennation angles for all training conditions. ***Significantly different from Pre ($p < 0.001$); †Significantly different from BFRT LV ($p < 0.05$)

The same researcher took all images. The researcher's reliability was assessed by the intraclass correlation coefficient (ICC). The ICC values were 0.976 ($p < 0.001$) for RF muscle thickness, 0.957 ($p < 0.001$) for VL muscle thickness and 0.916 ($p < 0.001$) for VL pennation angle. Ultrasound technique for the assessment of muscle architecture has previously shown this reliability values (7).

1RM assessment

During the familiarization session, as well as one week before the main experiment, subjects came to the laboratory to assess their concentric, bilateral, knee extension 1RM. The 1RM was considered as the maximum weight in kilograms that could be lifted only once in this particular piece of equipment. The test was carried out on an isotonic, monoarticular, open-chain leg extension machine (SuperGym, SG8019 Leg Ext/Hamstring Combo, Qingdao Impulse Group Co., Ltd., Mainland, China). After a standardized dynamic warm-up, subjects moved to the knee extension apparatus, where they performed a specific warm-up. The specific warm-up consisted of 8 repetitions with an intensity of 50% of their estimated 1RM. This set was followed by 2 minutes of rest and one set of 5 repetitions with an intensity of approximately 75% 1RM. Subjects were instructed to keep their arms crossed over the chest and avoid any extraneous movements of the hip and trunk during the test. This was done to minimize assistance of other muscle groups. Then, the load was set to an intensity of approximately 90% 1RM. Subjects were told to lift the load until volitional failure. A repetition was considered valid only if the subject used proper form and lifted the weight throughout the whole range of motion. If the subject managed to perform more than 5 repetitions the load was increased by 5% and the test was repeated after 3 minutes of rest. If they did not reach 5 repetitions, but they succeeded to complete more than one, the 1RM was estimated using the Epley's formula (15). On average, three trials were enough to complete the 1RM test. If necessary, another trial was performed after five minutes of recovery. 1RM values (mean ± SD) were as follows: 142.2 ± 20.8 kg (BFRT LV); 138 ± 29.9 kg (BFRT HV) and 147.9 ± 23.4 (HIT).

Statistical analysis

The normality of the data was checked and subsequently confirmed with the Shapiro–Wilk test. Two-way analysis of variance (ANOVA) procedures were used to determine changes between *groups* (BFRT LV, BFRT HV and HIT) over *time* (pre-post). DMS *post hoc* test was performed to determine main effects and interactions when significant F-values were detected. The level of significance was set at $p < 0.05$. Effect sizes were measured by partial Eta squared (η^2) for the ANOVA and also by Cohen's d for comparison between pre and post values. Cohen's d effect sizes were interpreted as follows: $d < 0.02$ = *null effect*; $d < 0.5$ = *small effect;* $d < 0.8$ = *medium effect;* $d > 0.8$ = *large effect.*

Results

Fascicle arrangement, as measured by pennation angle, tended to increase in all groups, though significant differences were only found in post following HIT ($p < 0.001$). HIT pennation angles at post were significantly higher than those of BFRT LV ($p = 0.013$) and BFRT HV ($p = 0.037$) (Fig. 1). *Post-hoc* interactions along with Cohen's d effects sizes are displayed in Table II.

Table II. Pre-post values of muscle architecture of rectus femoris (RF) and vastus lateralis (VL)

Group		RF (cm)		VL (cm)		Penn (º)		Cohen's d		
		Pre	Post	Pre	Post	Pre	Post	RF	VL	Penn
BFRT LV	Mean	22.7	25.8***	23.2	26.3***	15.9	17.0	1.36	0.82	0.32
	± SD	2.3	2.5	3.83	4.4	3.1	3.4			
BFRT HV	Mean	24.1	29.1***†	23.7	26.8***	16.2	17.5	1.82	1.43	0.90
	± SD	2.7	3.6	2.17	3.1	1.4	2.1			
HIT	Mean	24.7	29.1***†	23.8	25.3*	16.7	20.0***‡	1.70	0.49	1.09
	± SD	2.3	2.3	3.11	4.3	3.0	2.7			

***Significantly different from Pre ($p < 0.001$); *Significantly different from Pre ($p < 0.05$); †Significantly different from BFRT LV ($p < 0.05$); ‡Significantly different from BFRT LV and BFRT HV ($p < 0.05$). BFRT LV: low volume occlusive training; BFRT HV: high volume occlusive training; HIT: high intensity resistance training

All groups increased their RF muscle thickness values at post with respect to baseline (16.9%, $p < 0.001$, $\eta^2 = 0.869$). BFRT HV and HIT showed larger increases in RF muscle thickness as compared to BFRT LV ($p = 0.012$ and $p = 0.028$, respectively). VL muscle thickness was also increased in post, irrespectively of the training condition (10.9%, $p < 0.001$, $\eta^2 = 0.530$), with no further between-groups differences.

Discussion

Although the acute increases of muscle thickness following BFRT have been previously described (23, 28), this is the first study to examine variations in muscle thickness along with variations in fascicle arrangement. The main finding of the present study was that all groups showed an acute increase of muscle thickness, while only the HIT group fascicle arrangement was significantly affected.

Previous literature has shown that exercise affects muscle structure, usually increasing both muscle thickness and muscle fiber pennation angles. These variations of muscle architecture have been observed after exercises of different nature, ranging from pedaling to exhaustion (4) to medium intensity isotonic training (7) or maximal eccentric exercise (13). In our study all groups increased both RF and VL muscle thickness immediately after the cessation of the exercise. These results agree with those observed in other studies. After a protocol similar to that of the BFRT LV, Fry et al. (8) found leg circumference – an index of augmented muscle thickness – to increase immediately and 30 min after the cessation of the exercise. Similarly, other BFRT modalities, such as blood flow restricted walk training, have shown to immediately increase quadriceps muscle thickness after 30 min of treadmill walking at both slow (~6.7%) and high (~8.7%) speed (23).

Several mechanisms have been proposed to explain the acute post-exercise increase of muscle thickness, though they might differ between BFRT and HIT. An increase in vascular perfusion to the working muscles has shown to occur rapidly at the onset of HIT, mainly due to an increased need for oxygen and energy supply (26). Otherwise, an inflammatory response coming along with resistance training-induced muscle damage may explain, at least in part, the increase in muscle thickness observed after HIT (5, 22). On the other hand, increases in muscle thickness induced by BFRT have been associated with a loss of plasma volume (2) and augmented intramuscular pressure (27), which may result in a fluid shift from the vascular space into the blood flow restricted muscle (2). However, the low mechanical load used in BFRT studies has shown to induce mild to no change in indices of muscle damage (17, 18). It is also probable that the initial increase in muscle thickness when first applying the cuff was mostly due to venous pooling and not necessarily to a fluid shift into the muscle cells. However, at some point during the protocol it is probable that a fluid shift into the muscle cells did occur. If the acute change in muscle thickness was due solely to venous pooling, the muscle thickness value would have returned to baseline following the removal of the cuff.

Thus, it seems plausible that differing mechanisms underlie the increases in muscle thickness registered either after BFRT and HIT. In line with this, one of the limitations of the present study was that muscle thickness was only measured at the cessation of the exercise, so no data are available concerning recovery. Studying the differed response of muscle thickness could have helped us to ascertain whether inflammatory or perfusion reactions were responsible for the increase in muscle thickness.

In accordance with previous literature, HIT increased VL pennation angle immediately after the exercise (4, 7, 13). Surprisingly, changes in VL pennation angle were observed neither after BFRT LV nor after BFRT HV. Csapo et al. (7) attributed the increase of VL pennation angles after resistance training to an increased stiffness of tendons and aponeuroses. In support of this idea, it has been demonstrated that the stiffness of human tendon increases after resistance training (13, 25); furthermore, fascicle shortening has been associated with increased pennation angles (1). Thus, with increased tendon and aponeuroses stiffness, muscle fascicles will shorten more to maintain a given level of tension (7). This could explain the increased VL pennation angle observed following HIT. However, it has been demonstrated that tendon stiffness increases in a linear relationship with the training load. To illustrate, Kubo et al. (14) found that training with high internal muscle force induced greater increases in tendon stiffness compared with training with low internal muscle force. In support of this, chronic changes in tendon-aponeuroses stiffness have shown not to change following BFRT, while a group that trained with higher loads increased it significantly. For instance, it seems likely that in our study the mechanical tension was responsible for the increases in pennation angle of HIT group.

In summary, a high volume of BFRT induced an acute increase in RF and VL muscle thickness in a similar extent as HIT. These results support the hypothesis that acute muscle cell swelling induced by BFRT may be responsible for the processes underlying BFRT induced muscle hypertrophy. However, future research is needed to ascertain whether this acute increase of muscle thickness may be caused by muscle cell swelling or could reflect an inflammatory response. Additionally, pennation angles of VL only increased after HIT, suggesting that the higher mechanical load of HIT may have induced a response of the tendon-aponeuroses complex different to that of the BFRT groups. Thus, the overall response of muscle architecture to BFRT differs from that of traditional resistance training. This study provides an avenue on future research on the implications of these results to long-term muscle architecture adaptations induced by BFRT.

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