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The role of exercise selection in regional Muscle Hypertrophy: A randomized controlled trial

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ABSTRACT

There is emerging evidence suggesting that muscle growth is not homogeneous through the muscle. The aim of the present study was to analyse the role of exercise selection in regional hypertrophy. Two randomly allocated groups with equal training volume and intensity performed squats in the smith machine (SMTH group) or the leg extension exercise (LEG group). Growth in proximal, central and distal regions of the *rectus femoris* (RF) and *vastus lateralis* (VL) muscles, jump height and body composition were analysed. Results show that the three regions of RF grew significantly in the participants of the LEG group ($p < 0.05$), while only the central region of VL grew significantly in the SMTH group ($p < 0.05$). In summary, this study confirms that exercise selection plays a role in regional hypertrophy. Whilst there may be still other factors that determine how muscles grow, it seems that the chosen exercises may be responsible of the differences observed in this study.

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KEYWORDS
Inhomogeneous; quadriceps femoris; strength; growth; squat; leg extension

Introduction

Skeletal muscle hypertrophy can be defined as the increase in muscle fibre cross-sectional area (CSA) that is accompanied by an increase in muscle volume and mass. This muscle growth can occur in response to regular mechanical stimuli (Wackerhage et al., 2019), and it may not be homogeneous between the different heads of a muscle (Zabaleta-Korta et al., 2020). Muscle growth has also been shown to differ among the different regions of a single muscle head (Zabaleta-Korta et al., 2020).

This phenomenon is known as regional hypertrophy (Zabaleta-Korta et al., 2020) and the origin of this region-specific response to mechanical stimuli remains unknown. To date, it is not possible to establish a pattern of a region-specific growth in a muscle in response to different exercises. However, previous research suggests that eccentric movements imply an increased hypertrophy in the region closest to the knee in the *quadriceps femoris* (Franchi et al., 2014, 2018). Other authors have reported a regional increase in the muscle CSA at higher absolute intensities when compared to the same exercise at lower absolute intensities (Ema et al., 2013).

Recently, Costa et al. compared the growth of the *quadriceps femoris* when performing a single exercise *vs*. performing multiple exercises (Bd De V et al., 2021). The authors hypothesized that performing multiple exercises would make all the regions of the muscle grow to a similar degree, due to the varying stimuli, while regional hypertrophy would happen in the group that only did one exercise. However, regional hypertrophy was found in both groups, and consequently, the question arises whether this result was influenced by the exercises selected. Since one of the groups performed multiple exercises, this question cannot be answered with the data provided by this study.

The present study aimed to answer this question and to understand the implications of exercise selection for regional hypertrophy. We compared two different lower body exercises: the leg extension exercise (LEG) and the squat exercise performed in the Smith machine (SMTH). We measured the regional hypertrophic responses in different heads of the *vastus Medialis (VM)*, the *vastus lateralis (VL)* and the *rectus femoris (RF)*. We hypothesized that muscle adaptations would differ between both exercises. The leg extension exercise should elicit the growth of VM and VL and not RF, because of the lack of movement at the hip level, while the squat should make the RF and the rest of the muscle heads grow to a similar degree due to role of the RF as a hip flexor.

Methods

Subjects

Thirty-four healthy men (age = 25.4 ± 4.8 years; body mass = 76.3 ± 7.7 kg; height = 1.76 ± 6.5 cm; body fat percentage = 10.6 ± 2.1%; percentage of lean body mass = 89.4 ± 2.1%) with at least 2 years of resistance training experience joined this investigation. Participants were required to meet the following inclusion criteria: 1) men age ranging between 18 and 40 years; 2) lack of musculoskeletal disorders; and 3) experienced on resistance training defined as consistently lifting weights at least 3 times per week for 2 years. A total of 27 participants completed the study. Four participants dropped out because of a relative tested positive for SARS-COVID19, and

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three subjects dropped out for personal reasons not related to the study. Written informed consent was obtained from each participant after a thorough explanation of the testing protocol, the possible risks involved, and the right to terminate participation at will. The study was approved by the Institutional Review Board of the University of the Basque Country UPV/EHU (ref. 118/2019) and all procedures were done in accordance with the declaration of Helsinki (2013).

Procedures

Measurements were performed 2 days prior to the beginning of the training programme and 72–96 h after the end of it. Measurements lasted ~ 45′ and testing sessions were carefully scheduled to ensure that the same number of hours were left since the end of the training protocol. During the day of the first measurements, participants were randomly allocated into the SMTH and LEG groups. Participants in the SMTH group completed a 5-week resistance training programme consisting of 4 sets of 12 repetitions of deep squats on the smith machine three times per week taking each set to muscular failure. The participants of the SMTH group were trained to descend until the femur was parallel to the floor, or lower, in each repetition. Participants in the LEG group completed a 5-week resistance training programme consisting of 4 sets of 12 repetitions of the leg extension exercise taking each set to muscle failure. In this group, participants had to perform the larger range of motion possible. Participants in both groups were requested not to perform any other lower body resistance or power training, and they were also encouraged not to perform strenuous lower body activities during the period of the study. Weekly training volume consisted of 12 weekly sets. Baz-Valle et al. (2018) for the lower limbs and varied between 60 and 85 sets for the rest of the muscles (depending on the number of days that each subject trained).

Participants were allowed to perform upper body resistance exercises at will, and they were offered a standardized resistance training programme that involved both the corresponding lower body exercise and upper body exercises. Training was periodized in a flexible fashion: the first day, participants were told to adjust the weight so that they could reach muscular failure at 12 repetitions. For that purpose, participants were told to choose a weight that would make them reach but not exceed 12 repetitions on their exercise. If they were able to perform 1–3 extra repetitions with the chosen weight, they had to add 2.5 kg in the smith machine (or one more plate in the case of some leg extension machines). If they fell 1–3 repetitions short, they had to take 2.5 kg out of the smith machine (or a plate from some leg extension machines).

In subsequent weeks, participants were told to increase the weight every time they exceeded 12 repetitions with the current weigh. Weight increases were too high in the case of some leg extension machines, and for that reason some participants had to perform more repetitions to reach muscle failure. In the case of the leg extension exercise, if participants could perform more than 15 repetitions or if they were not able to reach nine repetitions with a given weight, they were trained to add or take out 1–2 resistance levels or plates.

As the duration of each of the leg extension phases has been reported to induce regional hypertrophyDiniz et al. (2020), in both groups, the eccentric phase of the exercise had to last 2″, and the concentric part of the exercise had to be performed as fast as possible. Participants were also requested to rest at least 48 h between training sessions. Resting between repetitions was not allowed, and participants had to rest from 3′ to 5′ between sets. Training sessions took place at the gyms in which each participant usually trained. To test whether the participants performed the exercises with the correct form, and with the right intensity, each participant had to send a video performing an effective set to the main researcher after every session. In order to be included in the study, participants had to complete at least 13 out of the 15 planned sessions, which accounts for 85% of the programmed training programme. Participants were given an excel spreadsheet to register the weights they lifted each session.

Anthropometric measurements

One day before and 3–4 days after the 5-week intervention, anthropometric characteristics of the participants were measured. Participants were weighed on a calibrated digital scale whilst wearing minimal clothing. Height was measured with a stadiometer attached to the scale with participants standing shoeless and head aligned in the horizontal Frankfurt plane. Eight-site skinfold measurements (in mm) were taken from the biceps, triceps, scapular, abdominal, suprailiac, thigh and medial calf sites according to standard procedures using a skinfold calliper (Harpenden1, Baty International, West Sussex, UK). All skinfolds were measured to the nearest 1 mm and the mean of three readings was recorded as the final value for each site. All body composition measurements were taken by the same investigator 24–48 h before and 72–96 h after completion of the training protocol. Body fat percentage was estimated using the equation proposed by Faulkner (1966).

Muscle size

Muscle size or Cross-Sectional Area (CSA) was measured using B-mode ultrasound imaging (GE LOGIQTM e, GE Healthcare, WI, USA) with a linear-array transducer (code 12 L-RS, variable frequency band 4.2–13.0 Mhz) (Hacker et al., 2016). Measurements were performed by an experienced technician with participants in a supine position, with arms and legs extended and relaxed. Prior to testing, participants remained in this position for 10 minutes to allow for stabilization of normal body fluids. The technician then applied a water-soluble transmission gel (Aquasonic 100 Ultrasound Transmission gel; Parker Laboratories Inc., Fairfield, NJ, USA) to each measurement site and a 9 MHz ultrasound probe was placed parallel to the tissue interface without depressing the skin. When the quality of the image was deemed as satisfactory, the technician saved the image to the hard drive and obtained CSA dimensions of the *vastus lateralis* (VL), *vastus medialis* (VM) and *rectus femoris* (RF) surrounding the muscles without including fascia or bone (Ruas et al., 2017) (Figure 1). Areas were measured using the polygon function of ImageJ software (Kim et al., 2020). Measurements were taken on the right side of the body at three different sites in the case of VL and RF. For VM, just two sites (25% and 50% of femur length) were considered because of its shorter nature. Measurements were taken at 25%, 50% and 75% of the distance between the lateral condyle of the femur and greater trochanter for the

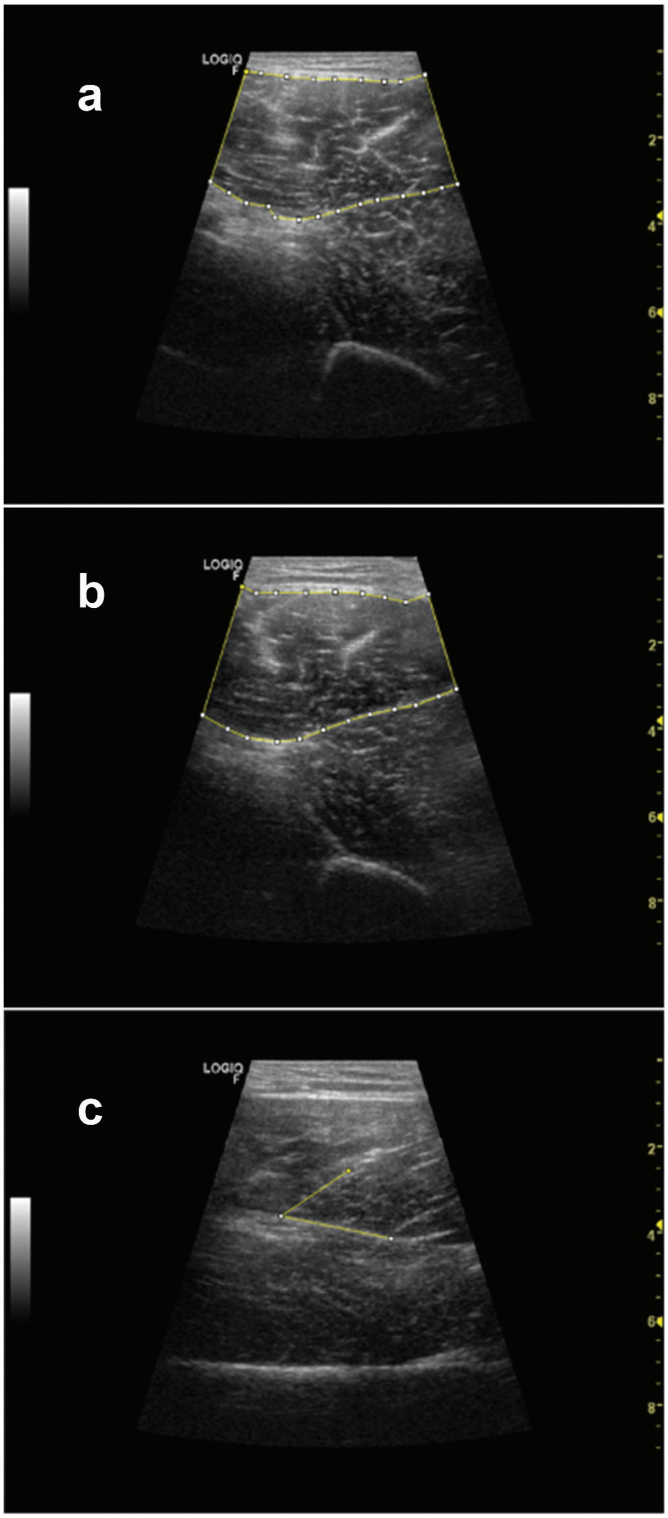

Figure 1. Measurement of the proximal region of the *rectus femoris* prior to the Intervention (A). Measurement of the proximal region of the *rectus femoris* after the intervention (B). Measurement of the pennation angle of the central region of the *rectus femoris* prior to the intervention (C).

central (RF) and lateral (VL) aspects of the thigh. Three images were taken at each site and the values were averaged to obtain a final measurement. In an effort to help ensure that swelling in the muscles from training did not obscure results, participants were told not to train 72 hours prior to the measurements. This timeframe is consistent with research showing that acute increases in muscle thickness return to baseline within 48 h following a resistance training session (Ogasawara et al., 2012).

Pennation angle

When the technician finished analysing the CSA of the area in which the pennation angle was going to be measured, the probe was placed perpendicular to the tissue interface without depressing the skin. When the quality of the image was deemed as satisfactory, the technician saved the image to the hard drive and obtained pennation angle of the central region of RF and VL and proximal region of VM by measuring the angle of the intersection between fascicle and the deep tendon aponeurosis (Figure 1).

Jumping performance

To measure jumping performance, participants performed three counter-movement jumps (CMJ) before and after the intervention period. After a standardized warm-up consisting of light aerobic cycling, active warm-up exercises and body weight squats, participants were taught how to correctly perform a CMJ. After 2 trials and a 3′ rest, participants performed three jumps that were measured by the Optojump photoelectric cells (Microgate, Bolzano, Italy). The photoelectric cells consisted of two parallel bars (one receiver and one transmitter unit, each measuring 100×4×3 cm) that were placed approximately 1 m apart and parallel to each other. The device was calibrated according to the manufacturer's instructions (Glatthorn et al., 2011). The best of three jumps was considered for further analysis. Participants were asked to maintain an eucaloric diet or to stay in a slight energy surplus (as explained in the next paragraph). In the absence of a power-oriented training, it was considered that improvements in the maximal jump height were caused by an increase in lower body strength.

Dietary adherence

To avoid the potential for dietary confounding, participants were given a document in which they were instructed to reach 2 $g{\cdot}kg^{-1}$ of protein intake. First, they were taught how to calculate how much protein they needed according to their bodyweight. Then, they were instructed on how to reach that amount with examples of different dishes and were requested to include at least 20 g of protein per meal divided into 3 to 5 meals with at least 3 hours between them. Participants were also trained to be in an eucaloric diet or slight energy surplus. Participants also agreed not to take any supplements that could interfere with the study outcomes (such as creatine or whey protein).

Statistical analyses

We tested all variables for normal distribution (Shapiro–Wilk test) and homogeneity of variances (Levene's test). An independent samples student *t*-test was used on pre-intervention muscle cross-sectional area and pennation angle data to check for potential differences between groups. A repeated measures ANOVA (group × time) was used to determine which of the regions (proximal, central or distal) had grown during the study and analyse the changes in weight, fat percentage and jump height both within and between subjects. Partial eta squared (n^2p) were calculated to analyse the magnitude of the potential pre-post intervention differences between participants (Olejnik and Generalized Eta 2003). The ES were interpreted as trivial (<0.01), small (0.01), medium (0.06) or large (0.14) (Goss-Sampson 2020). All calculations were performed using SPSS 26.0 for Mac. The level of significance was set at $p < 0.05$.

Results

No significant differences were observed between groups in any of the CSA or pennation angle variables analysed pre-intervention ($p > 0.05$). Analysis of the CSA of the VM was not performed because images were not clear enough to determine the area of the muscle head in 50% of the subjects.

Growth differences between regions

All the regions of the RF muscle grew significantly during the intervention ($p = 0.015$, $p = 0.022$ and $p < 0.001$; Table 1) in the LEG group, but no region reached statistical significance in the SMTH group. Regarding VL, only the central region of the participants of the SMTH group grew after the study ($p = 0.004$) (Table 1).

Growth differences between groups

When comparing the response of a single region to the two different exercises, no significant differences were found between groups ($p > 0.05$) and all effect sizes were small (<0.06) (Table 2).

Pennation angle

Pennation angle did not increase during the intervention ($p > 0.05$) (Table 3).

Anthropometrical characteristics and strength

Body mass, body fat percentage and jump height did not differ between groups before the intervention period ($p > 0.05$). However, after the intervention, the body mass ($p = 0.015$; 95% CI = −2.19-,0.28) and the jump height ($p = 0.01$; 95% CI = −2.41–0.39) of the SMTH group increased, while they remained unchanged in the LEG group ($p > 0.05$).

Table 1. Growth per regions in each group.

Muscle	GROUP	Region	CSA pre (cm^2)	CSA post (cm^2)	% change
RF	SMTH	25%	13.3 ± 1.7	13.7 ± 2.6	3.0
		50%	11.6 ± 2.2	11.8 ± 2	1.2
		75%	6.2 ± 1.6	6.0 ± 1.1	−2.2
	LEG	25%	13.5 ± 2.0	14.7 ± 2.6*	9.0
		50%	11.8 ± 2.0	12.8 ± 2.1*	8.8
		75%	5.9 ± 1.8	7.0 ± 1.9*	19.7
VL	SMTH	25%	15.1 ± 2.0	15.7 ± 2.4	4.1
		50%	16.2 ± 2.1	17.2 ± 2.5*	6.0
		75%	13.3 ± 2.7	13.4 ± 2.4	0.7
	LEG	25%	15.1 ± 3.0	15.6 ± 2.7	2.8
		50%	15.9 ± 2.4	15.8 ± 2.3	4.3
		75%	12.5 ± 2.6	13.3 ± 2.6	6.5

RF, *rectus femoris*; VL, *vastus lateralis*; CSA, cross-sectional area.
*Significantly different compared to PRE.
#Significantly different compared to the same region of the SMTH group.

Table 2. Comparison of a region between groups.

GROUP	Region	P	ES
RF	PROX	0.335	0.037
	MED	0.419	0.027
	DIST	0.310	0.043
VL	PROX	0.938	0.0002
	MED	0.173	0.073
	DIST	0.616	0.01

RF, *rectus femoris*; VL, *vastus lateralis*; ES, effect size.
*Significantly different compared to VL.
#Significantly different compared to RF.

Table 3. Increase in pennation angle in each group.

GROUP	Muscle	PA pre (deg)	PA post (deg)	% change
SMTH	RF	12.4 ± 3.8	12.7 ± 4	2.5
	VL	22.8 ± 5	21.4 ± 3.8	−5.8
	VM	21.8 ± 6.6	22.7 ± 4.9	4.0
LEG	RF	16.3 ± 5.5	18.2 ± 4.3	11.7
	VL	19.5 ± 4.1	20.6 ± 4.9	5.8
	VM	20.5 ± 4.9	21.7 ± 5.5	5.9

RF, *rectus femoris*; VL, *vastus lateralis*; VM, *vastus medialis*; PA, pennation angle.

Discussion

The main finding of this study was that exercise selection had a large influence in the regional growth pattern of the muscles analysed. In particular, the *rectus femoris* increased its CSA after an intervention using leg extension exercise, with the central and distal regions of this muscle head being the ones that grew the most. When performing squats in the smith machine, the central region of the *vastus lateralis* head was the one that grew the most, and the greatest improvement in jump height was also achieved in the group performing this exercise.

These results data are in agreement with previous research describing that leg extension induces the growth of the RF, in particular in the distal part (Ema et al., 2013; Hisaeda et al., 1996). However, the reason of this asymmetric growing patterns remains unclear. During this exercise, there is no movement at hip level, so we expected a greater growth of the VL and VM than the RF. The explanation behind this phenomenon may be the activation differences within the RF muscle. It is known that when performing a leg extension exercise, the distal part of the RF is activated to a greater extent (Watanabe et al., 2012). At the same time, when performing hip flexion exercises, the proximal part of the RF is also more active (Watanabe et al., 2012). This finding may also be a consequence of the biomechanical constraints of the quadriceps muscle. The moment arm length of the RF is maximal when the knee is totally extended (Visser et al., 1990), which is the hardest point for the athlete in the range of motion of the leg extension exercise. This, together with the higher distal activation of the RF in the knee extension tasks, may imply an extra stimulus to this muscle head. Some authors have suggested that this relationship between the forces within a muscle and the demands of the exercise are important when aiming at maximizing muscle growth (Walker et al, 2013).

Against this theory, we observed that the growth response of VL was not as large as in the RF. This makes sense in the case of the SMTH group as this exercise implies the maximum resistance when the knees are totally flexed and the moment arm of the VL is the smallest. However, in the leg extension exercise the largest moment arm of VL is at the total extension of knee joint. Surprisingly, the only exercise in which the VL grew was the squat in the smith machine, which according to this force/resistance hypothesis should not elicit a large effect to the VL. Therefore, the most probable cause of regional hypertrophy would be a combination of the demands imposed to the muscle and other factors more related to the disposition of the muscle to grow.

We cannot assure that regional hypertrophy does not happen by chance yet, as it may be caused may be variables like genetics, sex or age. Even if evidence fails to explain why the regional hypertrophy does happen, we still do not know whether it is a response to mechanical stimuli, or just the normal pattern of growth that a muscle may follow after any type of mechanical stimuli. Interestingly, animal models have already shown unequal adaptions in different regions of a muscle after the same stimuli, maybe due to the distinct architecture of each analysed region (Butterfield and Herzog, 2006). Previous research also suggests that there may be other factors that could influence the regional muscle hypertrophy responses. For example, a recent study reported that the speed at which each phase of an exercise is performed may modify the region-specific response of the quadriceps muscle (Diniz et al., 2020). These authors proposed that the time spent in each phase of an exercise change the torque at the knee joint and thus, this could be responsible for this regional response of growth. As strength gains are mode dependent (Paschalis et al., 2011; Margaritelis et al., 2021) it seems reasonable to think that muscle hypertrophy would also follow a mode dependent growth pattern. However, there may be other unknown factors playing a role in the regional hypertrophy responses of a muscle.

Finally, it is worth noting that participants in the SMTH group increased their vertical jump significantly while participants in the LEG group did not (Table 4). This can be easily explained by the specificity principle. The exercise that the SMTH group did for 5 weeks was much more similar to a vertical jump (involving both hip and knee extensions) compared to the one that the participants in the LEG group did (involving only knee extensions). The bodyweight of the subjects did not change from the beginning to the end of the study (Table 4), which shows that they were actually in a slight caloric surplus, and that improvements in vertical jump were due to the exercise they performed and not because of changes in their body mass.

Table 4. Changes in anthropometrical characteristics and strength during the intervention.

GROUP	Variable	PRE	POST	% change
SMTH	Body mass (kg)	76.9 ± 6.3	78.1 ± 6.5*	1.6
	Fat %	11 ± 2.4	12.9 ± 9.8	17.2
	CMJ (cm)	36.4 ± 4.8	37.8 ± 4.6*	3.9
LEG	Body mass (kg)	75.7 ± 9.1	76.5 ± 8.8	1.2
	Fat %	10.2 ± 1.8	10.2 ± 1.8	0.1
	CMJ (cm)	36.3 ± 5.5	36.4 ± 6.1	0.3

CMJ. countermovement jump.
*Significantly different compared to PRE.

Our study faced several limitations. First, the study lasted only 5 weeks, which can be considered short. Even if some studies confirm that a muscle begins to grow three weeks after the onset of an exercise program (Seynnes et al., 2007) we know that muscle growth would have been larger with a few extra weeks of training. However, considering that the study consisted in taking each set to muscular failure, it was designed to increase participants adherence to the study. Because of the duration of the study, we consider that participants of the SMTH group were in disadvantage to grow. Almost every participant in the SMTH group complaint for sore muscles during the first 3 weeks of training. Sore muscles, or delayed onset muscle soreness (DOMS), is an indicator of muscle damage, which has been reported to attenuate muscle growth (Damas et al. 2016). Therefore, part of the expected increase in muscle protein synthesis (MPS) caused by weight training in this group may have been used to compensate the muscle protein breakdown (Damas et al., 2016). This may explain, at least in part, the lesser growth of RF and VL of SMTH group after the intervention, although, since no direct indicator of muscle damage were measured, we cannot attribute the findings to muscle damage (Margaritelis et al., 2021).

Another limitation was that the weight increases were too large in the case of some leg extension machines, and for that reason some participants had to perform more repetitions to reach muscular failure. However, some authors found no difference on muscle hypertrophy when performing a different amount of repetitions as far as training volume and intensity were equated (Baz-Valle et al., 2019). For this reason, we consider that participants performing a few more repetitions (they never exceeded 16) had no influence on the outcomes of the study. It is worth noting that in the study, we used ultrasounds (US) instead of Magnetic Resonance Imaging (MRI), which is considered the gold standard. However, there is a strong correlation (rho = 0.88) between the CSA measured with MRI, and the one measured with US (Hacker et al., 2016). Lastly, even if participants were trained to consume enough protein and to maintain an eucaloric diet, they were not supervised. At the same time, supervision of the training sessions was performed with videos, which made the evaluation of certain technical aspects difficult. However, since almost every participant displayed some kind of growth in the *quadriceps femoris* muscle, we believe that these limitations did not interfere with the results.

In summary, this study confirms that exercise selection plays a role in regional hypertrophy. Whilst there may be still other factors that determine how muscles grow, it seems that the chosen exercises may be responsible of the differences observed in this study. Future research aiming at unveiling these factors and trying to determine their importance for regional hypertrophy is thus warranted.

Based on these findings, coaches and athletes may prioritize the leg extension exercise for sports where there is a high demand of the *rectus femoris* muscle (i.e. in soccer) (Mendiguchia et al., 2013). Similarly, bodybuilders who seek for homogeneity in the development of their lower limbs, should be encouraged to perform the leg extension exercise when aiming for RF hypertrophy. In contrast, to improve jumping performance or to address weakness of the VL, which may cause an imbalance between the power of VL and VM, and ultimately lead to injury (such as a displacement of the patella (Kapandji, 2010)), performing squats in the smith machine seems the best choice.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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