Effect of the contraction of medial rotators of the tibia on the electromyographic activity of vastus medialis and vastus lateralis

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A B S T R A C T

Purpose: This study attempted to assess if the resisted contraction of medial rotators of the tibia increases the ratio between the activity of vastus medialis (VM) and vastus lateralis (VL) during maximal isometric contractions (MIC) of the quadriceps femoral (QF) muscle at 90° of knee flexion.
Methods: About 24 female subjects participated in this study, performing four series MIC of the QF. In the first series subjects performed only MIC of the QF muscle, whereas in the other three there was MIC of the QF with resisted contraction of medial rotators of the tibia, with the tibia positioned in medial, neutral and lateral rotation. During each contraction, VM and VL electromyographic signal (EMGs) and QF force were collected, being the EMGs root mean square (RMS) used to access the activity level of these muscles. *Results:* The use of the General Linear Model (GLM) test showed that for $\alpha = 0.05$ there was a significant increase in the VM:VL ratio when the resisted contraction of medial rotators of the tibia was performed with the tibia in medial ($p = <0.0001$), neutral ($p = <0.0001$) and lateral rotation ($p = 0.001$). The same test showed that during MIC of the QF associated to resisted contraction of medial rotators of the tibia there were no significant differences in the VM:VL ratio between the three tibial rotation positions adopted ($p = 0.866$ [medial–neutral]; $p = 0.106$ [medial–lateral]; $p = 0.068$ [neutral–lateral]).
Conclusions: The resisted contraction of medial rotators of the tibia increases the VM:VL ratio during MIC of the QF and the tibial rotation position does not influence the VM:VL ratio during MIC associated to resisted contraction of medial rotators of the tibia.

Keywords: Resisted contraction of medial rotators of the tibia; Tibial rotation position; VM:VL ratio; Electromyography; Maximal isometric contraction

1. Introduction

Quadriceps dysfunction is a common problem found in rehabilitation, as most knee pathologies show vastus medialis (VM) atrophy (Haffajee et al., 1972). This may be because this muscle is the first to atrophy from disuse, and its recovery is slower, which causes less medial active force on the patella (Sakai et al., 2000; Powers, 2000). As a result, patellofemoral pain syndrome (PFPS) has been related to VM atrophy (Theodore, 1975; Gross, 1991; Karst and Willet, 1995; Sakai et al., 2000; Powers, 2000; Lohman and Harp, 2002; Manske and Davies, 2003). It has been shown that subjects with this syndrome present a VM activity lower than that of the vastus lateralis (VL), while normal individuals have a VM:VL ratio of 1:1 (Mariani and Caruso, 1979; Hanten and Schulthies, 1990; Gouilly and Jayuon, 2001; Earl et al., 2001).

Although all quadriceps portions contribute to patellar stability, VM seems to be the most influent due to its direct attachment on the superior and medial patellar borders and to the anatomic configuration of its fibers (50–55°) (Peeler et al., 2005). Although there is still some controversy as to VM's role (Peeler et al., 2005), it is argued that its primary function is to achieve patellar alignment (Lieb and Perry, 1968). Considering this role of VM in patellofemoral (PF) dysfunction cases, increasing this muscle's force is essential to ensure VM equilibrium as to the VL (Hanten and Schulthies, 1990; Mirzabeiji et al., 1999; Powers, 2000; Lee et al., 2002). This is consistent to the fact that VM strengthening can diminish lateral patellar displacement and the associated symptoms (Doucette and Goble, 1992; Sakai et al., 2000). As a result, the importance of studying ways of enhancing VM activity becomes clear.

Changes in VM and VL activation patterns have been associated to some factors such as knee flexion angle, hip adduction activity and tibial rotation. As to knee flexion angle, VM is active at all ranges, including at 90° (Mariani and Caruso, 1979; Minor, 1991; Sakai et al., 2000), where its activity is higher than VL's (Signorile et al., 1995; Lefebvre et al., 2006). There is evidence that these muscles present similar electromyographic activity in the final extension degrees (Mariani and Caruso, 1979; Karst and Jewett, 1993). Hip adduction activity seems to enhance VM activity as some VM fibers attach to the adductor magnus muscle, whose activation provides a more stable VM attachment. Tasks involving activity of hip adductors and knee extensors have been shown to be a good way to activate the VM (Hanten and Schulthies, 1990; Minor, 1991; Hodges and Richardson, 1993).

In spite of the controversy surrounding the issues mentioned above, tibial rotation is the one causing more discussion. Based on anatomical assumptions presented by Slocum and Larson (1968), many authors considered that the contraction of medial rotators of the tibia could change quadriceps femoris (QF) muscle pattern, facilitating VM activity (Gough and Ladley, 1971; Engle, 1987; Hanten and Schulthies, 1990; Minor, 1991; Signorile et al., 1995; Serrão et al., 2004; O'Sullivan and Popelas, 2005). While some authors suggest that VM activity is not related to medial rotation of the tibia (Duarte-Cintra and Furlani, 1981; Hanten and Schulthies, 1990; Signorile et al., 1995), there is evidence showing that the contraction of medial rotators of the tibia enhances VM activity (Laprade et al., 1998; O'Sullivan and Popelas, 2005). An explanation for the different results could lie on methodological procedures used to control: (A) knee flexion angle, (B) presence/absence of QF muscle extensor activity, (C) tibial rotation amplitude, (D) presence and intensity of the activity of medial rotators of the tibia.

From the studies above, and considering methodological differences adopted, some inferences can be made as to differences in results. Table 1 compares methodological procedures used in those studies and the results obtained. According to these, when the contraction of medial rotators of the tibia is resisted and isolated it requires non-selective activation of the QF muscle, without any differences between VM and VL (Hanten and Schulthies, 1990). However, when associated to isometric contraction of the QF muscle, the activity of medial rotators of the tibia promotes higher VM activation (Laprade et al., 1998). Nevertheless, these authors have adopted different tibial rotation positions, which does not seem to assume relevance in VM activity in closed kinetic chain exercises (Serrão et al., 2004). On the other hand, the combination of tibial rotators' activity and isometric contraction of the QF muscle does not promote differential QF activation if the contraction of medial rotators of the tibia is not resisted (Signorile et al., 1995). As a result, it seems that the contraction of medial rotators of the tibia only influences QF muscle activity if it is resisted and combined with isometric contraction of the QF.

The purposes of this study were: (1) to assess how the contraction of medial rotators of the tibia associated to maximal isometric contraction (MIC) of the QF muscle at 90° of knee flexion influences the VM:VL ratio; and (2) to assess if the tibial rotation position influences the VM:VL ratio during contraction of medial rotators of the tibia with MIC of the QF muscle.

2. Methods

2.1. Subjects

Twenty-four healthy female individuals were tested (age = 21.2 ± 1.95 years, body weight = 58 ± 5.13 kg, height = 1.63 ± 0.06 m and Q angle = 15.14 ± 0.79 degrees; mean ± S.D.), being excluded subjects presenting one or more of the following aspects: (1) recent osteoarticular or musculotendinous injury of the knee, knee movement limitations and presence of PF injury symptoms; (2) background and signs of neurological dysfunction which could affect lower limb motor performance; (3) history of knee surgery or prolonged use of corticosteroids (Buckwalter, 1995); (4) practice of a sports training program in the six months preceding the test, with a frequency of two or more days per week; (5) Q angle below 14° or above 17°; (6) cardiovascular or hypertension problems. Trials were performed using the dominant limb, which was identified by asking subjects to kick a ball (Keating and Matyas, 1996).

The study was conducted according to the institution ethical norms and conformed to the Declaration of Helsinki, dated 1964, being informed consent obtained from all participants.

2.2. Instrumentation

A Biopac Systems, Inc. – MP 100 Workstation™ (Biopac Systems, Inc. 42 Aero Camino Goleta, CA 93117) was used to collect all EMG data, which were sampled at 2000 Hz with a band-pass filter between 10 and 500 Hz, amplified (common mode rejection ratio (CMRR) >110 dB, gain = 1000) and analog-to-digital converted (12 bit). Data were collected on VM and VL using steel surface electrodes (TSD150), bipolar configuration, with a 11.4 mm contact area and an inter-electrode distance of 20 mm and a ground electrode. This equipment presents good reliability (intraclass correlation coefficient (ICC) = 0.80–0.91 for VM and ICC > 0.82 for VL), concerning the electromyographic signal (EMGs) root mean square (RMS) (Soderberg and Knutson, 2000) and validity (ICC = 0.13–0.45 (Pincivero et al., 2000)). A dynamometer (Globus Italia–via Vittorio Veneto 36–31013 Codogné-Italia) was used in the isometric mode to measure MIC (reliability: ICC = 0.97–0.98 (Bohannon, 1986)). Knee flexion angles and electrode angulation position were measured with a universal goniometer (Baseline; inter and intra-observer reliability: ICC = 0.97 and 0.98, (Trew and Everett, 2001); validity: ICC = 0.73–0.77 (Brosseau et al., 1997)). Time was monitored using a stop watch (Casio).

Some support material was used in this study: (1) Acqknowledge® version 3.8.1 to MP100 system; (2) HP DeskJet 930C printer; (3) Inter(R) Pentium(R) M, 1.60 GHz processor, 600 MHz; (4) quadriceps chair; (5) Rocher's cage; (6) stabilization bands; (7) alcohol and cotton; (8) abrasive pad; (9) Razor blades; (10) adhesive tape; (11) splint to stabilize the ankle in neutral position.

2.3. Procedures

2.3.1. Subject positioning

All subjects performed MIC in closed kinetic chain on the quadriceps chair, under the following criteria (Fig. 1): (i) hip and knee at 90° flexion; (ii) stabilization of the torso, the pelvis, right below the anterior superior iliac spine, and thighs (Pincivero et al., 2001); (iii) resistance applied 3 cm above the malleoli; (iv) stabilization of the ankle joint in neutral position with a splint (Gough and Ladley, 1971); (v) arms crossed over the chest.

Table 1
Methodological procedures adopted in studies that attempted to explore the influence of tibial rotation on VM activity.

Authors	QF isometric activity	Intensity of the activity of medial rotators of the tibia	Tibia rotation position/ knee flexion position	Preferential VM activity
Hanten and Schulthies (1990)	No	Contraction performed under external resistance	Neutral rotation/30° flexion	No
Laprade et al. (1998)	50% of maximal isometric force	Contraction performed under external resistance	30° lateral rotation/70° flexion	Yes
Signorile et al. (1995)	Maximal isometric force	Contraction performed without external resistance	Neutral, medial and lateral rotation/90° flexion	No
Serrão et al. (2004)	Maximal isometric force	Not explicitly activated	Neutral, medial lateral rotation/90° flexion	No

Fig. 1. This figure illustrates subject test position and electrode location.

2.3.2. Skin preparation and electrode placement

Skin surface was prepared to reduce electrical resistance to less than $5\ \Omega$ (Powers, 2000): (1) shave of VM and VL muscular belly area; (2) removal of dead cells with alcohol; (3) removal of non-conductor elements between electrode and muscle with abrasive pad (Turker, 1993; Soderberg and Knutson, 2000). The VM electrode was positioned 4 cm above the patella upper border and 3 cm measured medially and oriented 55° to a reference line drawn between the right antero-superior iliac spine and the center of the patella (Lieb and Perry, 1968; Hanten and Schulthies, 1990; Laprade et al., 1998; Pincivero et al., 2001; Serrão et al., 2004). The VL electrode was placed 10 cm above the patella upper border and 6 cm measured laterally, and oriented 15° to the reference line (Duarte-Cintra and Furlani, 1981; Serrão et al., 2004). Electrode locations were confirmed by palpation of the muscular belly with the subject in the test position, being the electrodes placed on the most prominent area (Signorile et al., 1995). The ground electrode was fixed to the patella center (Brown and Weir, 2001). Electrodes were fixed to the skin with adhesive tape, to avoid movement and to guarantee homogeneous and constant pressure. Between electrode positioning and beginning of EMGs collection there was an interval of 5 min (Basmajian and DeLuca, 1985).

2.4. Measurement of maximal isometric force of the QF muscle and EMG analysis

Following a warm-up consisting of three submaximal isometric contractions, each subject was instructed to perform four series of three trials each. In one of them they were asked to perform knee extension maximal force. In the other three series subjects were instructed to perform medial rotation of the tibia against manual resistance and knee extension maximal force. In each one of these three series the tibia was positioned in neutral rotation, maximal medial rotation and maximal lateral rotation. When performing medial contraction of tibial rotators, resistance was applied on the medial aspect of the foot (first metatarsal head). Simultaneously, counter-resistance was applied on the lateral aspect of the calcaneous, which helped maintaining neutral rotation, maximal medial rotation and maximal lateral rotation. All procedures and verbal encouragement were performed by the investigator, equally to all subjects (Brown and Weir, 2001). Measurements were randomized to reduce the order effect, which can be caused by fatigue and previous muscle activation.

The maximal isometric force of the QF muscle was collected three times, being each contraction maintained for 5 s and followed by a 2-min rest, confirmed by the non-existence of EMG activity (Brosseau et al., 1997; Brown and Weir, 2001). At the same time, EMG activity for each muscle was collected by a two-channel unit at 2000 Hz. The signals were pre-amplified at the electrode site and then fed into a differential amplifier with adjustable gain setting (12–500 Hz; CMRR: 95 dB at 60 Hz and input impedance of $100\ M\Omega$). The gain range used in the study was 1000 and signals were band-pass filtered between 20 and 500 Hz. Raw energy signals were digitized and stored on computer disks for subsequent analysis by the Acqknowledge software program, version 3.8.1. The signals collected within the first and last seconds of each 5 s of isometric contraction were not used for analysis because of the possible occurrence of knee movement at the initiation and completion of the test. Therefore, a 3-s window of EMGs was used for analysis. This window of raw EMG activity was processed using the RMS procedure to assess the electrical activity of each muscle. As there was an intrasubject data comparison and use of the ratio between VM and VL activity obtained during MIC, there was no need to normalize the signal. Following some authors (Signorile et al., 1995), we have used the average ratio between the VM and VL RMS and the maximal isometric force of the QF muscle, measured in the contractions performed in each trial.

2.5. Statistics

Data were analyzed with the software Statistic Package Social Science version 13.0. The sample was characterized by descriptive statistics.

Upon verification of the underlying assumptions, normality and sphericity, the General Linear Model (GLM) test was used with a 5% significance level ($\alpha = 0.05$), to assess (I) if there were significant differences in the VM:VL ratio of the QF muscle with and without resisted contraction of medial rotators of the tibia and (II) if there were significant differences in the VM:VL ratio in the three tibial rotation angles used during MIC of the QF muscle when associated to resisted contraction of medial rotators of the tibia. This test was also used to verify if there were significant differences in force values. It also became relevant to investigate, by the same test, if there were significant differences in the VM and VL EMGs RMS in the measures collected.

3. Results

Force values results obtained from the four series are presented in Table 2. The proof values in Table 3 show no significant differences ($\alpha = 0.05$) between series. As a result, the remaining data analysis can be done under the assumption that muscle contraction intensity was the same in all series.

Comparison of measurements taken without resisted contraction of medial rotators of the tibia (A) and with resisted contraction of medial rotators of the tibia (with the tibia positioned in medial

Table 2
Registration of mean, standard deviation, maximum and minimum values of force and EMG activity of VM and VL.

Measurements		N	Mean	Standard deviation	Minimum	Maximum
A	QF muscle force	24	31.13	±7.38	20.67	44.47
	VM RMS value	24	0.039	±0.0108	0.02	0.07
	VL RMS value	24	0.043	±0.0079	0.02	0.06
	VM:VL	24	0.914	±0.224	0.52	1.44
B	QF muscle force	24	30.74	±6.26	21.3	43.57
	VM RMS value	24	0.045	±0.0118	0.03	0.07
	VL RMS value	24	0.040	±0.0109	0.03	0.07
	VM:VL	24	1.172	±0.221	0.75	1.54
C	QF muscle force	24	30.1	±6.59	20.1	42.97
	VM RMS value	24	0.049	±0.0184	0.03	0.1
	VL RMS value	24	0.043	±0.0135	0.02	0.07
	VM:VL	24	1.166	±0.2646	0.75	1.7
D	QF muscle force	24	30.14	±5.84	19.53	38.37
	VM RMS value	24	0.047	±0.0148	0.03	0.08
	VL RMS value	24	0.043	±0.0092	0.02	0.06
	VM:VL	24	1.107	±0.2737	0.66	1.72

A – without resisted contraction of medial tibial rotators;
B – with resisted contraction of medial tibial rotators, tibia in medial rotation;
C – with, resisted contraction of medial tibial rotators tibia in neutral rotation;
D – with resisted contraction of medial tibial rotators, tibia in lateral rotation.

Table 3
Proof values (p) obtained in the GLM test for force intensity (N).

Compared measurements	Proof values (p)
A–B	0.522
A–C	0.115
A–D	0.118
B–C	0.277
B–D	0.314
C–D	0.947

A – without resisted contraction of medial tibial rotators.
B – with resisted contraction of medial tibial rotators, tibia in medial rotation.
C – with, resisted contraction of medial tibial rotators tibia in neutral rotation.
D – with resisted contraction of medial tibial rotators, tibia in lateral rotation.

(B), neutral (C) and lateral (D) rotation) allows verifying if the resisted contraction of medial rotators of the tibia influences the VM:VL ratio and the VM and VL EMGs RMS. Table 4 shows that there are significant differences in the VM:VL ratio obtained during MIC of the QF muscle with and without resisted contraction of medial rotators of the tibia. The VM:VL ratio was higher during MIC associated to resisted contraction of medial rotators of the tibia in all tibial rotation positions adopted. When muscle activity was analyzed separately, there was statistical evidence ($\alpha = 0.05$) showing that there was a significant increase in VM EMGs RMS, while there were no significant differences in the VL. Comparison of measurements taken with resisted medial rotation of the tibia with the tibia positioned in medial, neutral and lateral rotation allows studying the influence of the tibial rotation position in the VM:VL ratio and the VM and VL EMGs RMS. There were no significant differences in the VM:VL ratio obtained during MIC of the QF muscle with resisted contraction of medial rotators of the tibia in all tibial rotation ranges adopted: [medial (B), neutral (C) and lateral (D)]. Also, there was no evidence of the existence of significant differences in the activity of VM and VL muscles when analyzed separately.

Table 4
P values obtained in the GLM test.

Compared measurements	P value		
	VM	VL	VM:VL
A–B	0.015	0.195	<0.0001
A–C	<0.0001	0.998	<0.0001
A–D	0.001	0.855	0.001
B–C	0.178	0.158	0.866
B–D	0.626	0.081	0.106
C–D	0.308	0.881	0.068

A – without resisted contraction of medial rotators of the tibia.
B – with resisted contraction of medial rotators of the tibia, tibia in medial rotation.
C – with resisted contraction of medial rotators of the tibia, tibia in neutral rotation.
D – with resisted contraction of medial rotators of the tibia, tibia in lateral rotation.

4. Discussion

Our results corroborate other studies sustaining that tibial rotation favors a preferential activation of the VM. In fact, these findings show that the contraction of medial rotators of the tibia lead to an increase in the VM:VL ratio. They also confirm that this variation results from an increase in VM activity, as VL activity did not register significant differences. A literature review shows that tibial rotators activity, associated to 50% of MIC of the QF muscle, is responsible for an increase in the VM:VL ratio at 70° of knee flexion (Laprade et al., 1998). According to our results it can be concluded that the mechanism leading to the contraction of medial rotators of the tibia, responsible for an increase in VM activity, can also be observed at 90° of knee flexion. These results can be explained by the fact that at this knee flexion angle the VM acts as medial rotator of the tibia (Engle, 1987), as it inserts into the anteromedial aspect of the tibia through a medial extensor aponeurosis (Slocum and Larson, 1968). O'Sullivan and Popelas (2005) also found that the latest degrees of tibial rotation increase VM activity.

Most studies focusing on the isolated effect of contraction of medial rotators of the tibia did not found an increase in the VM:VL ratio (Duarte-Cintra and Furlani, 1981; Hanten and Schulthies, 1990). On the other hand, our results show that the combination of contraction of medial rotators of the tibia and isometric contraction of the QF muscle increase VM activity, which is consistent to conclusions from other studies (Laprade et al., 1998; O'Sullivan and Popelas, 2005).

Considering some studies (Engle, 1987; Slocum and Larson, 1968), it could be expected that an active and resisted isolated rotation would lead to differential VM activation, but this was not shown (Duarte-Cintra and Furlani, 1981; Hanten and Schulthies, 1990), which may be due to the high degree of QF force which is needed for an optimal activation of the VM (Pincivero and Coelho, 2000). From this, it can be concluded that the isolated contraction of medial rotators of the tibia does not require the same activity that is needed to selectively activate significant VM activity.

According to our results, at 90° of knee flexion, the variable 'tibial rotation position' does not influence VM activity. This is supported by other researchers who, although not activating the medial rotators of the tibia explicitly, achieved the same VM activity in the three tibial positions, medial, neutral and lateral (Serrão et al., 2004). Contradicting our study, according to Signorile et al. (1995) the neutral position (without medial rotation activity) is the one where VM activity is higher.

We used two independent variables: the activity of medial rotators of the tibia and the tibial rotation position. In line with authors defending that VM activity is higher in the neutral position (Signorile et al., 1995) we could expect higher VM activation in the measurements taken without resisted contraction of medial rotators of the tibia. The assumption that there must be a high degree of QF voluntary force for an optimal activation of the VM (Pincivero and Coelho, 2000) can explain this difference in results. From this we can suppose that there must be a high level of voluntary force of medial rotators of the tibia for a maximal activation of the VM, as this muscle can work as medial rotator of the tibia (Engle, 1987). This may be the reason why exercises with contraction of medial rotators of the tibia do not increase VM activity when compared to the VL (Signorile et al., 1995), while this increase occurs in resisted contraction exercises, as used in our study. While in our study all subjects performed several trials using a splint to stabilize the ankle at neutral position, as the activation pattern of the muscles under study changes according to the ankle joint position (Gough and Ladley, 1971), these authors (Signorile et al., 1995) asked subjects to just maintain the ankle joint in neutral position. As far as electrode placement is concerned, these authors were based only on muscle belly palpation, placing electrodes on the most prominent part of it, whereas we have also used standard electrode positioning based on electromyographic studies. In our study only females were examined, while these authors (Signorile et al., 1995) included both males and females.

From the considerations above, it seems that maximal levels of contraction are needed for an optimal VM activation. This can be explained by VM's muscle fiber type, by its cross-sectional area and force-generating ability when compared to the others portions of the superficial QF muscle (Salzman et al., 1993; Travnik et al., 1995). The great proportion of type I muscle fiber contained in the VM in relation to other portions of the QF muscle may be sub-served by a correspondingly greater proportion of low threshold motor neurons, and may also indicate that a higher level of descending drive may be necessary to recruit its' higher threshold motor neurons (Pincivero et al., 2000).

A knee flexion angle of 90°, although not indicated for exercises during the first rehabilitation stages, is mentioned as the best choice for isometric exercises in later rehabilitation stages (Signorile et al., 1995). Moreover, there is evidence that this knee flexion angle is the most common isometric position in the knee rehabilitation process, being the amplitude where the QF muscle is recruited more efficiently (Basmajian and DeLuca, 1985; Engle, 1987; Signorile et al., 1995; Sakai et al., 2000; Chan et al., 2001; Ahtiainen et al., 2003; Serrão et al., 2004).

Our results show that, as far as MIC of the QF muscle is concerned, the VM:VL ratio significantly increases with resisted contraction of medial rotators of the tibia, with the tibia in medial, neutral and lateral rotation. These premises show that the resisted contraction of medial rotators of the tibia increases the VM:VL ratio during MIC of the QF muscle. No significant differences were found in the VM:VL ratio measured with the tibia in the following positions: (1) medial and neutral rotations; (2) medial and lateral rotations; (3) neutral and lateral rotations. We can therefore conclude that the variable "tibial rotation position" does not influence the VM:VL ratio during MIC of the QF muscle associated to resisted contraction of medial rotators of the tibia.

Notwithstanding the fact that we only tested healthy subjects, the mechanism that explains the association between medial contraction of the tibia and VM activity is present both in healthy individuals and individuals with PF dysfunction. In fact there are no differences in the VM:VL ratio between healthy subjects and subjects with PFPS in exercises with contraction of medial rotators and knee extension (Laprade et al., 1998).

Although this study points to some conclusions on the influence of the resisted contraction of medial rotators of the tibia in the VM:VL ratio, there are still questions to be answered. For future research remains the question whether the resisted contraction of medial rotators of the tibia influences the VM:VL ratio in other knee flexion angles, namely at 30° and 60°, as these are, together with 0° and 90°, the amplitudes mentioned by some authors as preferential for isometric strengthening of the QF muscle in PFPS cases. It would also be interesting to investigate how the intensity of contraction of medial rotators of the tibia influences VM selective recruitment, as this variable was not measured in the studies mentioned above.

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