Between-muscle differences in coactivation assessed using elastography

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Abstract (192 words)

Purpose: This study aimed to assess muscle coactivation in quadriceps and hamstring muscles using ultrasound shear wave elastography.

Methods: During maximal voluntary isometric contractions (MVC), both myoelectrical activity and shear modulus of antagonist muscles were measured (i.e., *rectus femoris*, *vastus lateralis* and *vastus medialis* during knee flexions and *semitendinosus*, *semimembranosus* and *biceps femoris long head* during knee extensions). To account for changes induced by inevitable joint rotation during MVC, the shear modulus values were compared to those measured at the same knee angle during a passive cycle. The difference between these values was considered as coactivation.

Results: Myoelectrical activity was detected in all antagonist muscles (8.0 ± 4.9% of maximal EMG RMS). Significant differences were observed between shear modulus values measured during MVC and those measured at the matched knee angle for all muscles (range: 2.7-4.8 kPa; all $p < 0.011$) except for *semitendinosus* (+1.7 ± 5.0 kPa; $p = 0.16$) and *semimembranosus* (+1.2 ± 5.6 kPa; $p = 0.39$). The magnitude of coactivation varied greatly among individuals.

Conclusions: Although non-negligible myoelectrical activity was observed in all muscles, coactivation assessed using elastography was considered as negligible in both the *semitendinosus* and *semimembranosus*. Between-muscle and between-participants differences warrant further investigation.

Introduction

Coactivation is defined as the activation of an antagonist muscle that accompanies the contraction of an agonist muscle (De Luca and Mambrito, 1987, Enoka, 2015). It is mediated by the inhibition of Ia-interneurons which decreases reciprocal inhibition of motor neurons innervating antagonist muscles and by the modulation of recurrent inhibition of antagonist muscles (Crone and Nielsen, 1989, Nielsen, 2016). Coactivation is thought to increase the stability of the joint through regulation of muscle stiffness (Reeves et al., 2008, Sartori et al., 2015). Numerous studies have estimated the mechanical effect of coactivation through the relationship between myoelectrical activity measured using electromyography (EMG) and joint torque (Kellis, 1998). Substantial antagonist contraction has been reported for quadriceps and hamstring muscles during maximal concentric (hamstrings: ≈23.7% of maximal voluntary contraction [MVC] (Aagaard et al., 2000, Baratta et al., 1988), eccentric (quadriceps: ≈8.5% of MVC (Aagaard et al., 2000) and isometric contractions (hamstrings: ≈23% of MVC; quadriceps: ≈5% of MVC (Macaluso et al., 2002).

An important assumption made by the studies that quantified coactivation using surface EMG is that the recorded myoelectrical activity accurately reflects muscle activation. However, the myoelectric signal detected over the targeted muscle may originate, at least in part, from nearby muscles. This phenomenon is referred to as “crosstalk” (Farina et al., 2016, Winter et al., 1994). For example, although a significant surface EMG signal was measured in the *soleus* muscle during ankle dorsiflexion, leading to the conclusion that co-activation was occurring, no activation was detected through fine-wire electrodes (Etnyre and Abraham, 1988). The distance from the recording electrodes to muscle fibers, which is related to the thickness of the subcutaneous tissues, may influence the amount of crosstalk (Latash, 2018). For example, even though Wu et al. (2017) reported a greater amplitude of the surface EMG signal of the antagonist *biceps femoris* in males than females, this difference disappeared when accounting for the difference in adipose tissue thickness. Together, these results suggest that the mechanical effect of coactivation assessed using surface EMG might be overestimated.

Shear wave elastography (SWE) has been proposed as a possible alternative to identify the contribution of antagonist muscle (Raiteri et al., 2016). This technique provides an accurate estimation

of muscle stiffness during passive stretching and submaximal contractions, with the changes in muscle stiffness being closely related to the changes in muscle force (Brandenburg et al., 2014, Chernak et al., 2013, Hug et al., 2015). Taking advantage of this technique, Raiteri et al. (Raiteri et al., 2016) observed no significant changes in stiffness of the antagonist *gastrocnemius lateralis* muscle during maximal isometric dorsiflexion, despite substantial muscle activation (19.1 ± 12.9% of maximal EMG amplitude). This discrepancy between EMG results, which suggest the existence of a large coactivation, and elastography results, which suggest the absence of active coactivation, needs to be confirmed in other muscle groups.

Significant differences in coactivation may exist between joints and muscles (Frey-Law and Avin, 2013). For example, coactivation of knee flexors assessed using EMG reached coactivation levels of up to 35 ± 14% of MVC (Beltman et al., 2003) whereas antagonist muscle activity of 19 ± 13% were observed for plantar flexors (Raiteri et al., 2016). These differences may result from EMG recording conditions [e.g. the distance from the electrodes to the source and the resistance of tissues (Latash, 2018)], the nature of the task (Lavoie et al., 1997, Petersen et al., 1999) or variations in reflex pathways (Yavuz et al., 2018). This can result in different coactivation between muscle groups crossing the same joint, as reported for the knee joint where reciprocal inhibition is higher for hamstring than quadriceps muscles (Hamm and Alexander, 2010).

This study aimed to assess muscle coactivation in quadriceps and hamstring muscles. As proposed by Raiteri et al. (2016), we took advantage of ultrasound shear wave elastography to estimate the shear modulus (an index of stiffness) of the antagonist muscles. Because muscle shear modulus is strongly related to both active (Bouillard et al., 2012) and passive force (Hug et al., 2015, Maisetti et al., 2012), this approach allowed us to dissociate the active and passive components of coactivation (Raiteri et al., 2016). We compared these results to those obtained with a more classical assessment of coactivation using the measurement of EMG amplitude. We hypothesized that i) coactivation would be larger when assessed using EMG compared to elastography and ii) lower coactivation level would be observed for hamstrings than quadriceps.

Methods

Participants

Eighteen healthy volunteers (age: 24 ± 3 yr.; height: 1.78 ± 0.09 m; body mass: 65 ± 11 kg; 9 females and 9 males), with no recent history of musculoskeletal injury participated in this experiment. Participants were informed of the procedure before providing written consent. The study was approved by the ethics committee of Paris III (ref no. 3418) and the French Health Agency (IRB no. 2016-A00715-46). All procedures conformed to the standards of the Declaration of Helsinki.

Data collection

The experiment involved isometric contractions and passive cycles performed on an isokinetic dynamometer (Con-trex, CMV AG, Dübendorf, Switzerland) which assessed the torque produced by the participant. Torque signal was digitized by a 12-bit analog-to-digital converter (DT9804; Data Translation, Marlboro, MA, USA) at 1000 Hz, corrected for gravity and low-pass filtered at 20 Hz in the forward and reverse directions using a third order zero phase Butterworth filter.

During each test, the three-dimensional position of the leg and thigh was collected by a seven-camera optoelectronic motion capture system (Vicon Motion System Ltd, Oxford, UK). Briefly, each camera produced infrared light, reflected by the markers and then captured by these same cameras. The collection of the marker's position by at least two cameras enables the calculation of its 3D position by triangulation. Four reflective markers were attached to the lateral malleolus, the tibial head, the lateral femoral epicondyle and the greater trochanter. Models of the leg and thigh segment were created with the manufacturer software to calculate the knee angle. Marker position was sampled at 100 Hz and low-pass filtered at 20 Hz using a third order zero phase Butterworth filter.

Myoelectrical activity was recorded using wireless electrodes (Zerowire, Aurion, Italy) placed over the *rectus femoris* (RF), *vastus lateralis* (VL), *vastus medialis* (VM), *semitendinosus* (ST), *semimembranosus* (SM), and *biceps femoris long head* (BF) of the tested leg. B-mode ultrasound (Aixplorer, Supersonic Imagine, Aix-en-Provence, France) was used to accurately place the electrodes longitudinally on the muscle fascicle's alignment (for each muscle except RF), away from the borders of the neighboring muscles. Due to the complex architecture of the RF, the electrodes were placed in the shortening direction of this muscle. Before electrode application, the skin was shaved and cleaned

with alcohol. Ag/AgCl electrodes (Blue sensor N-00-S, Ambu, Copenhagen, Denmark) were attached to the skin with an inter-electrode distance of 20 mm (centre-to-centre) following SENIAM recommendations (Hermens et al., 2000). Raw EMG signals were pre-amplified (input impedance: 20 MΩ; common-mode-rejection ratio: 90 dB; signal-to-noise-ration: >50 dB; gain: 1000) and sampled at 2000 Hz (Zerowire, Aurion, Milan, Italy).

An Aixplorer ultrasound scanner (version 6; Supersonic Imagine), coupled with a linear transducer array (4–15 MHz, SuperLinear 15-4, Vermon, Tours, France) was used in shear wave elastography mode (musculoskeletal preset). In short, the linear transducer array produces a focused ultrasound beam. Each pushing beam generates a mechanical perturbation that results in the propagation of shear waves. An ultrasound imaging sequence is then performed to acquire successive raw radio-frequency data at a very high frame rate (up to 2000 Hz). One-dimensional cross-correlation of successive radio-frequency signals is used to determine the shear wave velocity (Vs) along the principal axis of the probe. This propagation velocity is directly related to the shear modulus of the tissue, that is, the faster the shear wave propagation, the higher the shear modulus.

$$\mu = \rho V_s^2$$

where μ is the shear modulus of the tissue, ρ is the density of the tissue (1000 $kg.m^{-3}$ for muscles) and V_s is the shear wave velocity. A 2-dimensional map of shear modulus is provided at one sample per second. The optimal ultrasound probe location and orientation was determined such that several fascicles could be observed for all muscles except for the RF, for which the ultrasound probe was aligned with the shortening direction of the muscle (Hug et al., 2014). These locations were marked on the skin using a waterproof marker so that the transducer location remained constant for all measurements. Previous studies from our group (Lacourpaille et al., 2012, Morales-Artacho et al., 2017) and others (Umegaki et al., 2015) have reported a good to excellent inter-session reliability of the shear modulus measurements for quadriceps (ICC: 0.74-0.87; CV: 4.7-5.6%) and hamstring muscles (ICC: 0.86-0.99; CV: 3.5-11.6%). Because there is a strong linear relationship between muscle shear modulus and muscle force, we considered changes in shear modulus as an index of changes in muscle force (see Hug et al. (2015) for a detailed review). A transistor-transistor logic

pulse originating from each system and recorded on the acquisition card was used to synchronize elastography, mechanical, motion capture and EMG data.

Experimental setup

Participants were positioned supine with the right leg attached to an isokinetic dynamometer arm (Con-trex, CMV AG, Dübendorf, Switzerland). The right hip and knee joints were flexed at 90° (0° = anatomical position and full extension for the hip and the knee, respectively). Non-compliant straps were used to secure participants position and minimize changes in hip angle throughout the contractions. This setup ensured a stable positioning of the ultrasound probe over the hamstring and quadriceps muscles during maximal isometric knee extension and flexion, respectively (Fig. 1).

Experimental tasks

After a specific warm-up in isometric condition, participants performed nine maximal isometric knee extensions and flexions such that three measurements of shear modulus and EMG per antagonist muscle were obtained. Each contraction was maintained 3 s separated by 3 min of rest. Instead of achieving MVC torque as fast as possible, participants were asked to progressively increase their torque up to MVC over 6 s to ensure constant alignment and pressure of the ultrasound probe over the muscle belly. The order of the tasks (i.e. knee flexion and extension) and muscles recorded was randomized.

Passive shear modulus was assessed twice for each muscle. Flexor and extensor muscles were passively stretched before measurements through five slow ($10°.s^{-1}$) loading/unloading cycles for conditioning purposes. Because subtle knee rotations occur during maximal isometric contractions, passive shear modulus was assessed in each thigh muscle during passive cycles performed at $1°.s^{-1}$ throughout a range of motion that encompasses this knee rotation (i.e., from 100° to 80° of knee angle). The order of tested muscles was randomized.

Data analysis

All data were processed using Matlab (Mathworks, R2015a, Natick, MA) custom-written scripts. Elastography recordings of each trial (passive cycle and MVC) were exported in video format and sequenced into images. Image processing converted the colored map into shear modulus values (Fig. 1A). The region of interest was inspected to exclude non-muscular structures and artifacts (saturated values and void areas).

All EMG signals were first band-pass-filtered in the forward and reverse directions (10-500 Hz, third-order zero phase Butterworth filter). A 100-ms sliding window with a 1-ms step was applied to EMG signals to calculate the root-mean square (RMS) amplitude. The maximal EMG RMS amplitude (EMG RMS_{max}) was the maximal value achieved during MVC when each muscle acted as an agonist. The EMG RMS of antagonist muscle was then normalized to this maximal EMG RMS value.

The 3-s torque plateau reached during each maximal isometric contraction was used for further analysis. The antagonist muscle shear modulus, EMG RMS and corresponding knee angle (Fig. 1A) were considered at the peak torque produced by agonist muscles. The shear modulus measured at the matched knee angle during passive cycle was determined from the passive shear modulus–knee angle relationship (Fig. 1B). This value was then subtracted from the shear modulus measured during maximal contraction to determine the shear modulus representative of active force produced by antagonist muscles only. EMG amplitude was continuously monitored to ensure that this relationship accounted for passive muscle force only. Note that no passive cycles were excluded given that EMG RMS was systematically lower than 3% of EMG RMS_{max}.

Statistics

All statistical analyses were conducted using Statistica version 7.1 software (StatSoft, Tulsa, OK). Normality testing (Kolmogorov-Smirnov) was consistently passed, and values are therefore reported as mean ± SD. For each muscle group, we ran a repeated-measures analysis of variance to compare EMG RMS among synergist muscles (within-subject factors: muscle). The significance level was set at $P < 0.05$. When required, *post hoc* analyses were performed using Bonferroni tests.

Due to technical limitations of the scanner in measuring high shear modulus values (Hug et al., 2015), measurements cannot be normalized to the value measured during MVC; in turn, between-muscle comparison of shear modulus values is meaningless. Therefore, paired t-tests were used to compare the shear modulus value measured during MVC and during the passive cycle at a matched knee angle to test whether active coactivation was present. The absence of a significant difference indicated that the change in shear modulus during MVC was only due to passive knee rotation and therefore active coactivation was negligible. The significance level was set at $P < 0.033$ using the Benjamini and Hochberg procedure (Benjamini and Hochberg, 1995) to limit the false discovery rate induced by multiple comparisons (n=6).

Results

EMG activity measured while muscles acted as antagonists ranged from 6.7 ± 4.2 % to 9.4 ± 3.9 %. No differences were found between muscles (main effect of muscle: $p = 0.84$ and $p = 0.69$ for Quadriceps and Hamstrings, respectively; Fig 2).

When considering the quadriceps during maximal knee flexion, shear modulus reached from 7.0 ± 2.7 kPa for RF to 12.4 ± 5.5 kPa for VL (Fig. 3A). Knee angle was 96.4 ± 3.2° at peak isometric torque achieved during MVC, leading to a mean passive shear modulus of 6.1 ± 2.0 kPa (Fig. 3A). Significant differences were observed between the passive shear modulus measured at matched angle and the value measured during maximal knee flexion for RF (2.8 ± 2.8 kPa: $p < 0.001$), VL (4.3 ± 5.3 kPa: $p = 0.003$) and VM (3.3 ± 4.9 kPa; $p = 0.011$).

When considering the hamstrings during maximal knee extension, the shear modulus reached from 9.0 ± 3.8 kPa for ST to 20.4 ± 7.1 kPa for BF (Fig. 3C). Knee angle measured during knee extension MVC was on average 83.8 ± 2.7°. The passive shear modulus measured at the corresponding angle reached 12.5 ± 6.8 kPa (Fig. 3C). There was a significative difference between the passive shear modulus assessed at matched angle and that measured during maximal knee extension for BF (4.8 ± 6.9 kPa, $p = 0.009$). In contrast, no differences were observed for ST (1.7 ± 5.0 kPa; $p = 0.16$) and SM (1.2 ± 5.6 kPa; $p = 0.39$). Note that a large variability was observed between participants (Fig. 3D).

Discussion

Taking advantage of shear wave elastography, this study aimed to assess the coactivation of quadriceps and hamstring muscles during maximal isometric contractions. Despite EMG data suggested a substantial and similar level of coactivation among antagonist muscles, elastography data provided evidence of between-muscle differences in coactivation. Specifically, all quadriceps muscles were consistently coactivated during knee flexions. Inversely, only BF muscle exhibited substantial coactivation when the hamstrings acted as antagonists. For ST and SM, no differences were observed between the shear modulus measured during MVC and that measured at rest at the corresponding knee angle. Because this difference is attributable to the active force produced by the muscle, it provides evidence that active coactivation is negligible for ST and SM. Our findings also show that the magnitude of coactivation differed between individuals, with potential functional consequences on joint stability.

Muscle activation measured in the present study using surface EMG is comparable to that reported by previous work (quadriceps: 4.5-11.9% of maximal EMG amplitude (Krishnan and Williams, 2010, Macaluso et al., 2002); hamstring: 5.3-12.7 % (Kellis and Katis, 2008, Krishnan and Williams, 2010)). However, Macaluso et al. (2002) observed higher values of coactivation in hamstring muscles (i.e., 23-41%). Although the origin of these differences is unclear, subcutaneous adipose tissue has been proposed as greatly influencing the amount of crosstalk from agonist muscles (Wu et al., 2017). Variations in adipose tissue thickness among muscles and participants might thereby affect the level of coactivation inferred from surface EMG. Here, we used elastography, which offers two main advantages: i) it provides a more direct estimation of muscle force than EMG; and ii) it is insensitive to crosstalk (Hug et al., 2015).

We considered co-activation as present when the shear modulus of the antagonist muscles measured during MVC and that measured during passive condition at a matched joint angle were significantly different (Raiteri et al., 2016). Using this approach, significant coactivation was observed for all quadriceps muscles (Fig. 3A). This result concurs with previous observations inferred from EMG data during maximal knee flexions (Krishnan and Williams, 2009, Saito et al., 2013). The presence of a quadriceps active force may be a prerequisite for maintaining joint stability during

maximal knee flexion (Baratta et al., 1988, Frey-Law and Avin, 2013, Kellis, 1998). More precisely, the contraction of thigh muscles has been reported as inducing a shear force on passive structures crossing the knee joint (i.e. anterior and posterior cruciate ligaments; (Frey-Law and Avin, 2013, Kellis, 1998)). Previous data demonstrated the ability of antagonist muscles to compensate for such internal loading forces that may induce joint instability (Solomonow et al., 1987). In addition, although the present maximal contractions were performed at the same angular position, they resulted in slight changes in joint angle (~6°). These small variations shorten the moment arm (Visser et al., 1990). Thus, quadriceps coactivation could contribute to counteracting the decrease of antagonist muscle mechanical advantage (Baratta et al., 1988).

When considering the hamstrings, a significant difference was observed between the shear modulus measured during MVC and that measured at matched knee angle for BF but not for SM and ST muscles (Fig. 3C). This absence of antagonist active shear modulus despite substantial myoelectrical activity is in line with previous observations that coactivation of plantarflexors is negligible during maximal isometric dorsiflexion (Raiteri et al., 2015, Raiteri et al., 2016). The present study provides further evidence that the EMG technique may overestimate coactivation during isometric contractions likely because of the presence of crosstalk.

Even though EMG data suggested that quadriceps and hamstring muscles were coactivated during maximal isometric contractions, the elastography technique showed different coactivation strategies for quadriceps and hamstring muscles. As coactivation of antagonist muscles is mediated by reflex responses, these differences could originate from specific reciprocal inhibition (Bayoumi and Ashby, 1989). Using electrical stimulation, Bayoumi and Ashby (1989) have demonstrated stronger reciprocal inhibition from knee extensors to flexors while the opposite was not true. Our findings could thus reflect larger reciprocal inhibition from quadriceps that may translate into substantial lower coactivation of hamstring muscles during maximal isometric knee extension.

Our results also demonstrate differences in coactivation between hamstring muscles with only BF being actively coactivated. Although previous studies reported significant coactivation levels for all hamstrings muscles, they also reported a larger EMG amplitude in lateral (BF) than medial (SM and ST) hamstring muscles (Aagaard et al., 2000, Krishnan et al., 2011). Such differences in

coactivation strategies may contribute to improve accuracy in tasks that require more precision, e.g. during upper arm movements (Gribble et al., 2003). An additional functional advantage derived from this strategy is that it may protect the passive structures of the knee joint by restricting internal tibial rotation and the associated shear force (Aagaard et al., 2000).

Inspection of individual shear modulus values reveals substantial variability between participants. For example, active shear modulus calculated in participant 3 was very close to 0 kPa, while it was much higher in participant 14 (7.6 kPa, 9.6 kPa and 5.1 kPa for ST, SM and BF, respectively; Fig. 3D). Although isometric contractions performed in the present study could be considered as a constrained motor task, muscular redundancy still exists, leading to different possible force-sharing strategies to achieve the motor task. Each individual may therefore use a different combination of muscles over time. The interindividual variability could thus reflect the existence of individual-specific force-sharing strategies to ensure dynamic stability of motor performance (Latash, 2018).

The causes of such differences are unclear. Additional force from antagonist muscles may result from an inadequate passive force from joint stabilizers (e.g., ligaments crossing the knee). For example, Solomonow et al. (1987) provided evidence that antagonist muscles (i.e., hamstrings) played a substantial role as a stabilizer in individuals who had a deficient anterior cruciate ligament. Alternatively, these differences could result from different force-generating capacities (i.e., physiological cross-sectional area [PCSA], specific tension and moment arm) among individuals. For example, a muscle with a larger PCSA may need lower activation to produce sufficient antagonist torque to stabilize the joint. Thus, despite the differences in shear modulus between individuals or muscles, the variation in antagonist muscle torque may be lower, if not lacking, due to differences in force-generating capacity. Further studies including separate groups of individuals who exhibit clear differences in coactivation strategies could more directly investigate this hypothesis.

The use of shear wave elastography as an index of muscle force during submaximal contractions require substantial considerations. Because the current ultrasound shear wave elastography technique cannot accurately measure the shear modulus of very stiff tissues, we were not able to normalize the shear modulus values to that recorded during MVC (Hug et al., 2015). Such a

normalization procedure is a prerequisite to quantify the mechanical effect of each muscle in the production of antagonist joint torque. In the absence of normalization, our results only provide information about the changes in muscle force between MVC and the passive condition (while the muscle acts as an antagonist, where an absence of change can be directly interpreted as an absence of active coactivation as for ST and SM muscles.

Conclusion

This study provides new insights into the coactivation strategy involved in quadriceps and hamstring muscles during maximal contractions. We first observed substantial and similar myoelectric activity for all muscles. However, the use of elastography revealed negligible coactivation in both the *semitendinosus* and *semimembranosus*. In the latter case, the antagonist torque is mostly the result of an increase in passive force. This suggests that the level of coactivation varies between muscles and that surface EMG cannot detect such differences, likely due to crosstalk.

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Figure captions

Fig. 1: Illustration of the signal processing employed to determine the level of *the biceps femoris long head* coactivation during maximal knee extension. A. Typical raw data of antagonist shear modulus collected on *biceps femoris* muscle and variation of knee angle during maximal knee extension. B. Typical raw data of shear modulus and variation of knee angle during a passive cycle. Quantification of the active part of shear modulus determined by subtracting the amount of passive shear modulus to MVC shear modulus at knee match-angle and direction.

Fig. 2: Coactivation level assessed with EMG. Mean ± SD values are presented for quadriceps (*rectus femoris* [RF], *vastus lateralis* [VL], *vastus medialis* [VM]) (A) and hamstrings (*semitendinosus* [ST], *semimembranosus* [SM] and *biceps femoris* [BF]) (B).

Fig. 3: Muscle shear modulus during maximal voluntary contraction (MVC), passive cycle and the subsequent active part, with significant differences between conditions for quadriceps [*rectus femoris* (RF), *vastus lateralis* (VL), *vastus medialis* (VM)] (A) and *biceps femoris* (BF) while no differences were observed for *semitendinosus* (ST), *semimembranosus* (SM) (C). Individual values of the active part of shear modulus are presented for quadriceps (B) and hamstring muscles (D).