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TITLE PAGE

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Title:

Quantification of muscle co-contraction using supersonic shear wave imaging

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ABSTRACT

Muscle stiffness estimated using shear wave elastography can provide an index of individual muscle force during isometric contraction and may therefore be a promising method for quantifying co-contraction. We estimated the shear modulus of the lateral gastrocnemius (LG) muscle using Supersonic Shear wave Imaging and measured its myoelectrical activity using surface electromyography (sEMG) during graded isometric contractions of plantar flexion and dorsiflexion (n=7). During dorsiflexion, the average shear modulus was 26 ± 6 kPa at peak sEMG amplitude, which was significantly less ($P=0.02$) than that measured at the same sEMG level during plantar flexion (42 ± 10 kPa). The passive tension during contraction was estimated using the passive LG muscle shear modulus during a passive ankle rotation measured at an equivalent ankle angle to that measured during contraction. The passive shear modulus increased significantly ($P<0.01$) from the plantar flexed position (16 ± 5 kPa) to the dorsiflexed position (26 ± 9 kPa). Once this change in passive tension from joint rotation was accounted for, the average LG muscle shear modulus due to active contraction was significantly greater ($P<0.01$) during plantar flexion (26 ± 8 kPa) than at sEMG-matched levels of dorsiflexion (0 ± 4 kPa). The

negligible shear modulus estimated during isometric dorsiflexion indicates negligible active force contribution by the LG muscle, despite measured sEMG activity of 19% of maximal voluntary plantar flexion contraction. This strongly suggests that the sEMG activity recorded from the LG muscle during isometric dorsiflexion was primarily due to cross-talk. However, it is clear that passive muscle tension changes can contribute to joint torque during isometric dorsiflexion.

Keywords: gastrocnemius, antagonist muscle force, stiffness, shear modulus, elastography.

INTRODUCTION

Elastography techniques have been used to determine the localized *in vivo* mechanical properties of various tissues, including muscles. In particular, ultrasound shear wave elastography has proven useful in characterizing both active and passive dependent stiffness changes of muscle. It estimates the shear modulus from calculations based on the propagation velocity of remotely induced shear waves (Bercoff et al., 2004; Yamakoshi et al., 1990). *Ex-vivo* experiments have demonstrated that the muscle shear modulus is linearly related to the muscle Young's modulus measured using traditional material testing (Eby et al., 2013). Using one such imaging method, Supersonic Shear wave Imaging (SSI), a strong linear relationship between muscle shear modulus and individual muscle force has been reported in humans during isometric contractions [at joint torques up to maximal voluntary contraction (MVC), (Ateş et al., 2015)] and in *ex-vivo* chicken muscles during passive stretching (Koo et al., 2013).

Because of the strong linear relationship between muscle shear modulus estimated using SSI and both active and passive muscle force, SSI can provide an index of individual muscle force (Hug et al., 2015). Therefore, it may be a useful technique for quantifying co-contraction during isometric contractions. In a recent report (Raiteri et al., 2015), we used conventional B-mode ultrasound imaging to show that there was negligible co-contraction of the each of the *triceps surae* muscles during maximal isometric dorsiflexion, despite recorded antagonist surface electromyography (sEMG) root-mean-square (RMS) amplitudes of 12-23% MVC. We concluded that the antagonist sEMG signal was primarily due to cross-talk, based largely on the observed lack of fascicle shortening. However, quantification of fascicle shortening cannot provide an accurate quantification of passive and active muscle forces. Here we took advantage of SSI to provide a more direct assessment of active and passive force indexes associated with co-contraction during maximal isometric dorsiflexion. It is particularly important to develop approaches that can assess active and passive forces of antagonist muscles to understand how this impacts on torque generation at different joints, without relying on sEMG, which is susceptible to cross-talk (De Luca and Merletti, 1988). We hypothesized that the lateral gastrocnemius (LG) muscle shear modulus estimated during isometric dorsiflexion would primarily be associated with increases in passive tension induced by ankle rotation and that the active force contribution from co-contraction would be negligible.

METHODS

Eight recreationally active male subjects, aged 28 ± 5 (mean $\pm$ standard deviation) years, height 182 ± 8 cm and body mass 79 ± 9 kg, with no recent (<12 months) history of lower

limb injury, surgery or pre-existing neuromuscular disorders, participated in the study after providing written informed consent. The study protocol was approved and endorsed by the local University Ethics Committee and was conducted in accordance with the Declaration of Helsinki.

Subjects were seated in a reclined position with the left knee fully extended, the left ankle at 90° and the plantar aspect of the left foot flush against a custom-built rigid footplate, which had a force transducer attached to measure ankle torque. After pre-conditioning the muscle-tendon unit with five submaximal plantar flexion contractions (1-s hold, 1-s rest, ~80% MVC) and two MVCs of plantar flexion and dorsiflexion (3-s hold), subjects performed four alternating ramp contractions (10-s to target, 2-s hold) of plantar flexion (30, 35, 40 and 100% MVC) and dorsiflexion (100% MVC) with one minute of rest in between. Passive ankle dorsiflexions at ~1°/sec were executed by the investigator, through a range larger than the range measured during the contractions, to determine the passive shear modulus at multiple ankle angles.

Three-dimensional position data of the foot and shank was collected at 120 Hz using a four-camera optoelectronic system (Natural Point Inc., OptiTrack, Tracking Tools) to quantify movement of the foot and shank during the contractions and passive rotations (Raiteri et al., 2015). Muscle activity of the LG was recorded with sEMG using two electrodes (8 mm recording diameter, Ag/AgCl, Covidien, Mansfield, MA) placed just distal to the most prominent muscle bulge in a bipolar configuration with an inter-electrode spacing of 2 cm (centre to centre). EMG signals were amplified 1000 times

(Neurolog System, Digitimer Ltd., Letchworth, Hertfordshire) and band-passed filtered between 10-500 Hz, prior to being sampled at 2 kHz.

An SSI ultrasound scanner (Aixplorer, Supersonic Imagine, v. 8.0, Aix-en-Provence, France) with a linear transducer array (4-15 MHz, SL15-4) was used to estimate the LG muscle shear modulus at 1 sample/s during the contractions and passive rotations. The musculoskeletal preset was used with the optimization, persistence and smoothing parameters set to penetration, off and 4, respectively. The transducer was secured to the skin on the left shank over the LG (mid-belly) using adhesive bandage, which limited transducer movement relative to the skin. The final transducer location was where the clearest image of continuous muscle fascicles and aponeurosis could be imaged (Narici et al., 1996).

All data was processed using custom-written scripts in Matlab (Mathworks, R2014b, Natick, MA). The B-mode images and shear modulus color maps [pre-calculated in the Aixplorer software by multiplying the squared shear wave velocity by the muscle tissue density (1000 kg/m^3), (Ateş et al., 2015)] were exported in 'mp4' format and sequenced into 'jpeg' images. Image processing converted each pixel of the color map into a value of shear modulus based on the recorded color scale (Ateş et al., 2015; Hug et al., 2015). A rectangular region of interest (ROI) was defined as the largest muscle area within the color map that avoided aponeurosis and unfilled regions within the elasticity map. If any pixel of the shear modulus map within the ROI was saturated ($>266 \text{ kPa}$), which rarely occurred during dorsiflexion contractions and passive rotations, then the average shear

modulus was not calculated from that image and the ROI was redefined for that trial to eliminate saturated values. For sEMG measurements, a 100-ms sliding window was moved 1-ms at a time to calculate the RMS amplitudes of the LG muscle across each trial. The background signal (1-s average from the start of the trial when subjects were instructed to relax) was subtracted from the remaining signal to determine muscle activity during the contractions and passive rotations. Passive trials with sEMG RMS amplitudes of greater than 3% MVC were excluded from analysis.

During dorsiflexion, the LG muscle shear modulus and corresponding ankle angle were selected when the sEMG RMS amplitude peaked. This is because this point represents the peak effect considering the linear relationship between muscle isometric force, shear modulus and sEMG (Bouillard et al., 2011; Nordez and Hug, 2010). When the LG muscle acted as an agonist during plantar flexion, the shear modulus and respective ankle angle were selected at a sEMG-matched level. Because of unavoidable ankle rotation during the isometric contractions, the ankle angles taken above were matched to the same ankle angles during a passive rotation and then the respective passive shear modulus values were determined. These passive tension estimates were subtracted from the shear modulus estimated during the respective contractions to provide the shear modulus representative of active tension only.

Data was tested for normal distribution using the Shapiro-Wilk normality test. Differences in the LG muscle shear modulus estimated during plantar flexion and dorsiflexion were assessed using a paired t-test for normally distributed data or the

Wilcoxon signed rank test for data that was not normally distributed. Statistical analyses were completed using commercially available software (Prism 5; La Jolla, CA, USA). Data are represented as mean±standard deviation and the significance level was set as $P \leq 0.05$. Indexes of the effect sizes are reported using Cohen's d for normally distributed data or r for data that was not normally distributed.

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RESULTS

Only data from seven subjects was analysed, with data from one subject being excluded because their maximum dorsiflexion during the passive rotations was less than that during the dorsiflexion MVCs. Normalized sEMG RMS amplitudes of the LG showed peak activations of $19.1 \pm 12.9\%$ MVC during isometric dorsiflexion. The LG muscle shear modulus was significantly greater ($Z = -2.37$, $P = 0.02$, $r = 0.63$) during isometric plantar flexion (42 ± 10 kPa) than isometric dorsiflexion (26 ± 6 kPa) at sEMG-matched levels (Figure 1). The passive tension that contributed to the shear modulus during the contractions (Figure 1) was significantly less ($t(6) = 4.55$, $P < 0.01$, $d = 1.72$) during plantar flexion (16 ± 5 kPa) than dorsiflexion (26 ± 9 kPa) because of unavoidable ankle rotation, peaking at $5 \pm 3^\circ$ in the plantar flexion direction and $4 \pm 2^\circ$ in the dorsiflexion direction. After subtracting the passive tension estimates to quantify the shear modulus resulting from active force only at sEMG-matched levels (Figure 1), a significantly greater ($t(6) = 8.20$, $P < 0.01$, $d = 3.10$) LG muscle shear modulus was observed during isometric plantar flexion (26 ± 8 kPa) compared to isometric dorsiflexion (0 ± 4 kPa).

DISCUSSION

This study used ultrasound shear wave elastography to quantify passive and active force indexes (as estimated by the shear modulus) of an antagonist plantar flexor muscle during isometric dorsiflexion and then compared this to the force values predicted based on sEMG-matched levels when the same muscle acted as an agonist (isometric plantar flexion). The shear modulus measured at these matched levels increased during isometric dorsiflexion, however this increase was equal to the increase in passive shear modulus that resulted from ankle rotation. This result indicates that there was negligible active force generated by the LG muscle during isometric dorsiflexion, despite a measured increase in sEMG activity. This supports our previous assertions that the antagonist LG sEMG signal is primarily due to cross-talk contamination (Raiteri et al., 2015). However, our results also demonstrate that antagonist muscle force did increase during isometric dorsiflexion due to passive lengthening of the antagonist. This work provides the foundation for revisiting co-contraction during various tasks.

Because muscle stiffness changes may result from both passive and active tension changes, it is essential that the passive tension contributing to shear modulus estimations during contraction are adjusted for to give an accurate representation of stiffness dependent changes from contraction only to quantify co-contraction. The average LG muscle shear modulus estimated during isometric dorsiflexion demonstrates that this muscle was producing force when it acted as an antagonist. However, the passive tension produced by the LG muscle [which was similar to those reported for the LG (Basford et al., 2002) and MG muscles (Hug et al., 2013) in similar postures] was significantly

greater in the dorsiflexion contractions compared to the plantar flexion contractions. We believe that this was because of passive muscle lengthening resulting from joint rotation in the direction of the contraction. While there is some evidence that the shear modulus for active and passive loading is not equivalent at the same torque (Chernak et al., 2013), we are confident that our estimations of the LG muscle shear modulus were due to increased passive tension resulting from passive fascicle lengthening due to rotation of the ankle, and not co-contraction. This is supported by additional data showing that there was no difference in the fascicle length ($t(6)=0.05$, $P=0.96$, $d=0.02$) or pennation angle ($Z=-1.52$, $P=0.19$, $r=0.41$) when comparing the dorsiflexion contraction to the passive rotation at an equivalent ankle angle (see Supplementary Table 1). Therefore, while shear wave elastography is potentially a very informative tool for investigating antagonist muscle co-contraction, it is important to consider potential changes in passive contributions due to joint rotation.

The peak antagonist muscle activations we measured were similar to previous reports for the LG muscle (Elder et al., 2003; Tedroff et al., 2008) and to our previous study (Raiteri et al., 2015). Our shear modulus results suggest that this is not indicative of co-contraction (as classically interpreted), but rather due to cross-talk from dorsiflexor muscles, providing evidence that sEMG is not a good measure of antagonist muscle force during isometric contractions. However, our results do suggest that there may be changes in passive forces from the antagonists, due to joint rotation, and that these forces should be considered when attempting to estimate muscle forces based on external joint torque measurements that assume that all forces are generated by the agonists.

In conclusion, using SSI to estimate passive and active muscle force indexes, we have provided evidence that LG co-contraction during isometric dorsiflexion was negligible, despite emanating sEMG, and that increases in shear modulus were primarily because of passive tension increases resulting from unavoidable joint rotation. This work provides the foundation for revisiting co-contraction by considering both passive and active mechanisms contributing to antagonist muscle force.

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Disclosures:

The authors have no conflicts of interest, financial or otherwise, that might lead to potential bias.

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Figure 1. Active and/or passive tension of the lateral gastrocnemius (LG) muscle (as estimated by its shear modulus) during isometric plantar flexion and dorsiflexion contractions at surface electromyography (sEMG) root-mean-square (RMS) amplitude-matched levels determined at peak sEMG RMS amplitude during isometric dorsiflexion. Because of passive and active tension contributing to the shear modulus during the contractions (white bars), the passive tension of the LG muscle was predicted by estimating its passive shear modulus at equivalent ankle angles as in the sEMG RMS amplitude-matched contractions using a passive rotation (grey bars). This passive tension was subtracted from the shear modulus measured during the contractions to determine the active tension contribution to the measured shear modulus (black bars). Data are presented as mean+standard deviation. *: $P \leq 0.05$ for plantar flexion versus dorsiflexion for all three measures.

Net

Passive

Active

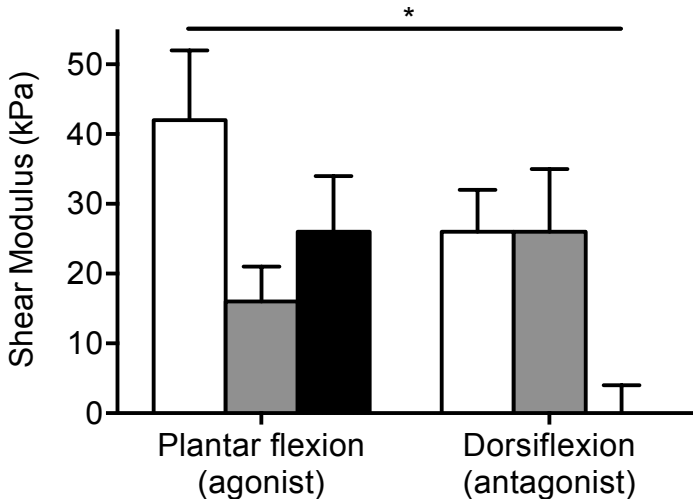


Figure 1.