HETEROGENEITY OF PASSIVE ELASTIC PROPERTIES WITHIN THE QUADRICEPS FEMORIS MUSCLE-TENDON UNIT

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Key words: elasticity; elastography; stress-shielding; tendinopathy; patellar; knee **Abbreviations:** electromyograhy (EMG), rectus femoris (RF), shear wave velocity (SWV), vastus lateralis (VL), vastus medialis (VM)

Running Title: QUADRICEPS MUSCLE-TENDON ELASTIC PROPERTIES

Acknowledgements: The authors thank Melanie Plinsinga for assistance with data extraction.

Funding source: Dr Coombes is in receipt of a Postdoctoral Fellowship for Women from The University of Queensland. No funding was received for the study itself.

Conflict of Interest: No conflict of interest, financial or otherwise, are declared by the authors.

Original research

Word count 2466

ABSTRACT

Purpose The purpose of this study was to compare regional elastic properties between anterior and posterior regions of the patellar tendon, and individual quadriceps muscles, over a range of knee flexion angles.

Methods An isokinetic dynamometer passively positioned the non-dominant knee of 19 young, healthy participants, at 25°, 40°, 55°, 70° and 85° flexion. Shear wave velocity (SWV, an index of tissue elasticity) was measured using ultrasound shear wave elastography in a relaxed (passive) state, confirmed by electromyography.

Results SWV of the patellar tendon and quadriceps muscles increased with knee flexion (longer muscle-tendon unit; P<0.001). Within the proximal third of the patellar tendon, SWV was lower in the posterior than anterior region at 70° (P=0.002) and 85° (P<0.001), but not at 25°, 40° or 55° (region-by-angle interaction, P =0.007). No differences were found between anterior and posterior regions within the middle third of the patellar tendon (P=0.332). For the quadriceps muscles, a significant muscle-by-angle (P <0.001) interaction was also observed. SWV of VL was greater than VM at 55° (P=0.005), 70° (0.17 [P=0.001) and 85° (P<0.001), but not at 25° or 40°. RF SWV was lower than VL at all angles (all P<0.002) and lower than VM at 55°, 70° and 85° (all P<0.002).

Conclusions Passive knee flexion at and beyond 70° was associated with non-uniform elastic properties within the proximal patellar tendon and between individual quadriceps muscles. To what extent this heterogeneity of passive elastic properties contributes to injury remains unknown.

INTRODUCTION

In vivo biomechanical properties of muscle and tendon have been classically inferred from inverse dynamics or measurements of joint torque using dynamometers and ultrasound measurement of tissue displacement (Hug et al. 2015). However, these measures provide information about the combined behaviour of several structures acting around a given joint and cannot isolate the behaviour of an individual muscle or isolated region within a tendon. There is growing evidence that differential passive strain may occur within tendons, such as the patellar (Almekinders et al. 2002), Achilles (Lyman et al. 2004) and supraspinatus tendons (Bey et al. 2002), which has been proposed to contribute to the development localised tendinopathy (Pearson et al. 2014). Likewise, heterogenous passive behaviour of synergist muscles, such as within the hamstrings muscles (Le Sant et al. 2017) might reflect the prevalence of localised muscle strain.

With the advent of innovative shear wave elastography techniques, it is now possible to estimate the mechanical properties of regional areas of soft tissues, including muscle (Koo et al. 2013) and tendon (Haen et al. 2015). The shear wave elastography technique uses acoustic radiation force to generate shear waves within tissue. Ultra-fast ultrasound imaging is used to measure the shear wave velocity (SWV) within tissues, which is positively related to tissue shear modulus, i.e. an index of stiffness (Bercoff et al. 2004). Measurement of muscle shear modulus (using elastography) has been shown to be strongly linearly related to both passive and active muscle force (Hug et al. 2015). Recently, shear wave elastography was used to examine shear modulus of the gastrocnemius muscle and Achilles tendon (Hug et al. 2013) and of individual quadriceps muscles (Xu et al. 2016) during passive ankle or knee movements respectively. Concurrent investigation of the quadriceps muscles and regions within the patellar tendon has not been undertaken.

Anatomical studies (Basso et al. 2001, Andrikoula et al. 2006, Hansen et al. 2006) describe the patellar tendon as comprising superficial (anterior) fascicles, which primarily extend from the rectus femoris muscle and pass over the anterior surface of the patellar; and deep (posterior) fascicles, some of which attach to the posterior surface of the patellar's inferior pole. Anterior fibres are longer than their posterior counterparts and both regions merge as they approach the tibial tuberosity (Basso et al. 2001). The ability to accurately quantify the mechanical properties of isolated regions of the musculotendinous unit will provide a more integrated understanding of passive muscle-tendon biomechanics. This information is important for both basic and clinical sciences and may advance our understanding of localised musculotendinous injury.

The objectives of this study were to 1) determine the reliability of localised shear wave velocity (SWV) measurement (within day and within rater); 2) compare SWV between anterior and posterior regions of the patellar tendon, and between the superficial quadriceps muscles (vastus medialis (VM), vastus lateralis (VL) and rectus femoris (RF)); and 3) correlate SWV values beween muscle and tendon regions; over a range of knee flexion angles (25°, 40°, 55°, 70° and 85°). We hypothesised that knee angle would affect the regional SWV within the proximal patellar tendon and individual quadriceps muscles.

MATERIALS AND METHODS

Participants

Twenty one healthy participants who were free of musculoskeletal injury in the back or lower limb in the preceding six months were recruited from The University of Queensland student community by flyers and word of mouth. Participants were asked to refrain from exercising on the day of testing. This study was approved by The University of Queensland's Human Research Ethics Committee and all participants provided written informed consent. Two participants were unable to fully relax their quadriceps muscles, as confirmed by surface electromyography (EMG) recordings, and were excluded shortly after commencing testing. Nineteen participants, aged 19 to 24 years (10 males, 9 females, age: 21.8±1.4 years; height: 171.5±9.4cm and weight: 66.1±10.3kg), completed the testing.

Experimental equipment

For the duration of testing, participants were seated in an isokinetic dynamometer (Kin Com, model 125AP, Chattanooga, USA), with the backrest declined by 30° from upright. All testing was undertaken on the non-dominant leg (dominant leg defined as the leg they would use to kick a ball). The dynamometer axis was aligned with the lateral epicondyle of the test leg (Maffiuletti et al. 2007) and the tibial strap was fixed at a distance of 20cm from this location. Chest straps were applied to limit trunk movement and foam wedges placed under the lateral thigh used where necessary to maintain neutral hip rotation. Using a manual goniometer, a reference point of 0° knee extension was calibrated on the isokinetic dynamometer, and the test-angles of 25°, 40°, 55°, 70° and 85° of knee flexion were determined.

Ultrasound and elastography examination was conducted using an Aixplorer Ultrasound Scanner (version 9.3; Supersonic Imagine, Aix-en-Provence, France) with a linear transducer (4-15MHz, Vermon, Tours, France) by a single examiner with 18 months of elastography imaging experience. Standard musculoskeletal muscle and tendon presets were used. The upper limit (16.3m/s) of the system was adopted for measurement of tendon SWV. Images were captured at 1.8 images/s for muscle and 1.6 images/s for tendon.

With the knee positioned at 70° knee flexion, B-mode ultrasound was used to identify and align the probe longitudinally to the fascicles of the patellar tendon, VM, VL and one compartment of the bipennate RF. Note that, of the muscles tested, the alignment with muscle fascicles is most difficult for the RF where a more complex muscle architecture can be observed within the B-mode image (Ema et al. 2013). For RF, the angle of the probe was tilted relative to the skin, to align with the three dimensional fascicle arrangement (Ema et al. 2013). Approximate positions of ultrasound and elastography imaging measurements were: 33.3% of thigh length for VM, 50-75% thigh length for RF and 33-50% thigh length for VL. The probe locations were drawn on the participant's skin with a waterproof marker to facilitate consistency in the repeated measurements. EMG surface electrodes (Trigno, Delsys, UK) were placed over each of the tested muscles, within 2cm proximal of the probe locations. Before electrode placement, skin was prepared with abrasive gel (NuPrep, Weaver & Company) and cleaned with alcohol. EMG recordings were used to provide real-time feedback to confirm that participants quadriceps muscles were relaxed throughout the experiment. Elastography images were discarded and the trial repeated if there was any evidence of concurrent EMG activity for any of the muscles, and as such EMG data is not discussed further here-in.

Procedure

As loading history affects tendon and muscle properties (Haraldsson et al. 2005), standardised preconditioning consisting of 10 passive cycles (0-85° knee flexion, 30°/s) was performed prior to each (of two) sets of measurements. Then the knee was passively moved to the following angles 25°, 40°, 55°, 70° and 85° (in this order), where two ten second elastography videos were recorded over VM, followed by RF, VL and then the patellar tendon. The order of testing was not randomised. Care was taken to minimise probe pressure during recording periods. Each set of measures took 12-15 minutes to complete. The average SWV measure, calculated from sets one and two, was used for further analysis.

In order to examine the within-day / within rater reliability of each measure, six participants were asked to return to the laboratory 60-90 minutes after completion of the first trial, where the entire protocol was repeated. Participants were free to move around the University grounds, but were asked to avoid strenuous activity for the period between tesing sessions. The location for recording ultrasound images (i.e. the probe locations) was marked on the skin, and the dynamometer position was recorded to facilitate set-up consistency between trials.

Data Extraction

Elastography videos were converted into images and customised MATLAB (R2016a, Mathworks Inc., Natick, MA, USA) scripts were used to extract the mean SWV in m/s for each series of images. The examiner was blinded for knee angle and participant by randomisation of the order of image processing. Regions of interest were manually determined using the B-mode image as visual guide (Fig. 1). The proximal one-third of the patellar tendon, originating at the distal edge of the patella's inferior pole, was evenly divided into anterior and posterior regions. Similarly, the middle third of the patellar tendon was evenly divided into anterior and posterior regions. For the quadriceps muscles, a representative area within the elastogram, that avoided overlying facia or areas void of colour was selected (Fig. 2). Mean±SD ROI areas (cm^2) were: 0.22±0.06 and 0.20±0.05 for the anterior and posterior proximal patellar tendon; 0.24±0.05 and 0.24±0.05 for anterior and posterior middle patellar tendon; and 1.18±0.22; 1.04±0.29 and 1.33±0.27 for VM, RF and VL respectively.

Statistical analysis

Intraclass coefficients (ICC) and 95% CI were calculated for anterior and posterior regions within the proximal patellar tendon and each muscle using linear mixed effects regression models, with angle as a fixed factor. Standard error of measurement (SEM) and coefficient of variation (CV) (typical error expressed as a percent of the participant's mean score) were also calculated as measures of within-day reliability (Hopkins 2000).

Linear mixed-effects regression models were used to examine the effect of region (anterior and posterior tendon) and angle (25°, 40°, 55°, 70° and 85°) on SWV measured within the proximal and middle thirds of the patellar tendon. Region and angle were entered as fixed factors and individuals and angle as random intercept and random slope, respectively. Differences in SWV between individual quadriceps muscles was also examined, substituting muscle (VM, RF and VL) for region in the model. Different within subject covariance models were considered, and Akaike information criteria and Bayesian information criteria were used to select the best fitting model. An unstructured covariance pattern was selected for tendon and an independent covariance pattern was selected for muscle. Model residual plots were checked to ensure the assumptions of the model were satisfied. Post hoc pairwise comparisons were performed using Bonferroni-adjusted *P*-values.

Analyses were performed using Stata 13.1 (StataCorp, USA) and two-tailed tests were considered significant if $P<0.05$. Data are presented as mean±SD or mean difference (MD) and [95% CI] as appropriate throughout the results section. Correlations between muscle and tendon regions SWV were visualised graphically and Pearson correlation coefficients are presented.

RESULTS

The within-day ICC and CV for measurement of SWV for tendon and each muscle ranged from 0.88 to 0.95 and 3.2% to 6.4% respectively (Table 1).

A significant effect of angle ($P<0.001$), region ($P<0.001$), and region-by-angle interaction ($P=0.007$) was observed for SWV within the proximal third of the patellar tendon (Table 2; Fig. 3). There were no differences in SWV between anterior and posterior parts of the proximal patellar tendon for more extended angles (25°, 40° and 55°). In contrast, significantly lower SWV was detected for the posterior proximal region than anterior proximal region at 70° and 85° (all $P\leq0.002$). For the middle third of the patellar tendon, a significant effect of angle ($P<0.001$), but no region or region-by-angle interaction was found. All mean differences between tendon regions and their 95% CI data are reported in Table 2.

For SWV within the quadriceps femoris muscle group, a significant effect of angle, muscle, and angle-by-muscle interaction (all $P<0.001$) was observed (Table 2, Fig 4). No differences in SWV were found between 25° and 40° for any muscle ($P>0.18$), or between 40° and 55° for RF. SWV increased after 40° in the monoarticular muscles (VM and VL) and after 55° for the biarticular RF ($P<0.04$). No differences were detected between VL and VM at 25° or 40° ($P>0.784$), however VL was greater than VM at higher knee flexion angles (55° to 85°, all $P<0.005$). RF was significantly lower than VL at all knee angles and lower than VM at 55°, 70° and 85° (all $P<0.002$). All pairwise mean differences between muscles at each knee angle and their 95% CI data are reported in Table 2.

Significant correlations were found between the anterior proximal patellar tendon and individual quadricps muscles, with Pearson correlation coefficients being higher for the monoarticular muscles than RF (VL r=0.74; VM r=0.61; RF r=0.45; *all* $P<0.001$). Correlation coefficients between the posterior proximal patellar tendon and the respective muscles (VL r=0.66; VM r=0.54; RF r=0.38; *all* $P\leq 0.001$) were lower than for the anterior counterpart.

DISCUSSION

Our first observation that knee angle influenced the distribution of shear wave velocity between anterior and posterior regions within the proximal third of the patellar tendon, aligns with previous cadaveric and in vivo work. By inserting strain gauges within the patellar tendon of eight fresh frozen limbs, Almekinders et al (2002) demonstrated similar tensile strain behaviour between regions with the knee in full extension (0°). During knee flexion, strain increased on the anterior side, while a relative decrease was observed on the posterior side of the proximal tendon (full extension was designated as the reference point). No regional differences were found at a more distal tendon location at any angle. In another study of 16 healthy volunteers, regional patellar tendon strain was examined with the knee fixed at 90° flexion using an ultrasound based automated pixel tracking technique (Pearson et al. 2014). Participants performed a series of ramped isometric knee extension contractions, ranging from 10% to 100% of maximal force. They demonstrated lower strain for the posterior compared to anterior region of the proximal patellar tendon, a pattern which was observed across force levels (Pearson et al. 2014). Extension of this work in ten healthy males was recently published (Pearson et al. 2017), demonstrating that during maximal ramped isometric knee extension, the anterior to posterior strain ratio was greatest at 90° knee flexion (7.8%) and lowest at 30° (2.9%) of flexion. Interestingly, these ratios (generated during maximal isometric contraction) are similar to those for our study which measured SWV in a relaxed state (1.1% at 25°, 2.2% at 40°, 3.0% at 55°, 5.6% at 70° and 8.4% at 85°).

Our second observation that knee angle influenced the distribution of shear wave velocity (and therefore of shear modulus) of both monoarticular (VM and VL) and biarticular (RF) quadriceps muscles is only partially supported by the literature. In a recent study of nine healthy males, a different pattern of muscle shear modulus was observed between monoarticular and biarticular muscles, but no differences were observed between monoarticular muscles throughout the range of knee flexion. In contrast, we demonstrated greater shear modulus for VL than VM at 55°, 70° and 85°. Of note, this between muscle difference was not observed when tested in extended knee positions, which corresponds to when the quadriceps muscles are below their slack length (Xu et al. 2016). Given a strong relationship exists between muscle shear modulus and muscle force (Hug et al. 2015) and because VL cross-sectional area is larger than that of VM (Hug et al. 2015), these findings provide strong evidence that knee flexion induces a larger increase in VL than VM passive force. Differences in hip position between our study (measured in hip flexion) and that of Xu et al, 2016 (measured in hip extension) may have influenced the relative stiffness of the biarticular RF compared to mono-articular muscles. Spatial variation within muscles (Le Sant et al. 2017) and orientation of the probe with muscle fascicles may have also differed between studies.

To our knowledge, this is the first study to perform concurrent investigation of the elastic properties of individual components of the quadriceps-patellar musculotendinuous unit. When tested in the current position (with the hip flexed), muscle-tendon SWV correlations were greater for the monoarticular muscles than the biarticular RF. Correlations were consistently greater for the anterior proximal patellar tendon than for its posterior counterpart. We postulate that this may be because fascicles from the anterior proximal tendon are more continuous with the quadriceps muscles. Our results also suggest the slack angle (angle at which there is a significant rise in passive SWV) may differ between muscle and tendon, as well as between monoarticular and biarticular quadriceps muscles (~25° for tendon; ~40° for VM/VL; ~55° for RF). The former result is consistent with a study measuring SWV of the gastrocnemius-achilles musculotendinous unit (Hug et al. 2013), which found a lower slack angle for tendon. The latter finding differs from Xu et al (2016) who observed the slack angle was similar among the quadriceps muscles (41-44° knee flexion), when tested in supine lying with the hip extended (placing greater stretch on RF).

The shear wave elastography technique overcomes some limitations of traditional measures by non-invasively estimating the shear wave propogation velocity within individual muscles and isolated regions of tendon. A distinct advantage is that it provides a real time elastogram, enabling visualisation of tissue regions of greater SWV (represented by red) and lower SWV (represented by blue). However, there are some limitations to this approach. Our within-day ICC was lower for RF (0.88), compared to VM and VL (0.95), which may be because of difficulty in aligning the probe with the muscle fascicles within RF. Probe orientation relative to fascicle direction may have also contributed to lower SWV values for RF (Aubry et al. 2013). For measurement of tendon SWV, our ICCs (0.92-0.93) indicated acceptable repeatability, while regional differences within the proximal patellar tendon (~8.4%) exceeded the typical error values (5.7-6.4%), highlighting the potential utility of the method to study regional in vivo patellar tendon biomechanics.

Although the relationship between SWV and shear modulus is well known for muscles, it remains unkown for tendons. Indeed, the guided wave propogation that occurs within tendon is known to affect the estimation of tissue shear modulus from SWV measurements (Hug et al. 2015, Helfenstein-Didier et al. 2016). However, the good relationship between tendon shear modulus estimated through guided wave propagation and tendon shear modulus measured using the shear wave dispersion technique that accounts for guided wave propagation (Helfenstein-Didier et al. 2016) makes us confindent that the differential changes in SWV reported herein represent true differencial changes in shear modulus. Scanning depth has also been shown to have an effect on SWV recorded from both elasticity phantoms and from muscle, having a greater impact on stiffer tissue such as the tendon (Carlsen et al. 2015, Ewertsen et al. 2016). However, we did not find a significant difference between anterior and posterior regions within the middle third of the patellar tendon, suggesting that scanning depth alone does not fully account for the regional differences observed within the proximal tendon. Importantly, only relatively low loads can be applied to tendon before the upper limit of 16.3m/s (Aixplorer Ultrasound Scanner, version 9.3) is reached, limiting current measurement of tendon stiffness to passive conditions only. While tendon stiffness during contraction would be expected to be greater by an order of magnitude than during passive conditions, the relative difference between anterior and posterior regions (~8%) does not appear to differ between maximal isometric contraction and passive conditions. Finally, although we performed passive preconditioning prior to each set of recordings, stress-relaxation may have affected the measurement of stiffness at successively greater knee angles (Haraldsson et al. 2005), which were not randomised in this study.

Our sample included young, active students without knee injury. Further extensive studies are needed to address the intriguing possibility that differential muscle and tendon passive elastic properties might contribute to the development of injury. “Stress-shielding” of the posterior relative to anterior patellar tendon has been proposed as an aetiological factor in tendon pathology (Pearson et al. 2014), which is typically located in the deep parts of the proximal tendon insertion onto the patellar (Johnson et al. 1996, Khan et al. 1996). Non-uniform tendon behaviour during knee flexion might result in shear type stresses and potentially underpin why volleyball athletes who display greater knee flexion angle during jump landing are more likely to have symptomatic patellar tendinopathy (Richards et al. 1996).

An imbalance of force generation between VM and VL has also been speculated to contribute to the development and/or persistence of patellofemoral pain (Hug et al. 2015), which is typically noted by patients when the knees are more flexed (Tang et al. 2001). Greater passive stiffness (and thus passive force) for VL than VM in knee flexion may be necessary to accommodate heterogenous muscle activation observed between VM and VL (Tang et al. 2001). Given the biomechanics of the patellofemoral joint are controlled by active and passive components, future studies may wish to consider concurrent measurement of muscle stiffness using shear wave elastography, muscle activation using EMG and muscle architecture to assess the balance of forces between muscles in people with and without patellofemoral pain (Hug et al. 2015).

This study benefits from usage of a highly innovative ultrasound shear wave elastography technique that allows for novel insights into the regional heterogeneity of passive mechanical properties within the quadriceps femoris muscle-tendon unit. Such insights are critical for future biomechanical models and research to quantify changes in muscle or tendon stiffness in clinical populations.

Table 1. Within-day reliability for shear wave velocity for each region within the proximal patellar tendon and quadriceps muscle.

Location	ICC (95%)	SEM (m/s)	CV (%)
Anterior patellar tendon	0.92 (0.84, 0.96)	0.58	6.4
Posterior patellar tendon	0.93 (0.86, 0.97)	0.47	5.7
Vastus medialis	0.95 (0.90, 0.98)	0.07	3.2
Rectus femoris	0.88 (0.77, 0.94)	0.10	4.8
Vastus lateralis	0.95 (0.90, 0.98)	0.09	3.8

Intraclass coefficients (ICC), calculated using a linear mixed-effects regression model. Standard error of measurement (SEM) and Coefficient of variation (CV) calculated per Hopkin (Hopkins 2000).

Table 2. Results of linear mixed-effects regression models for patellar tendon and quadriceps muscle shear wave velocity.

Model effects	**Comparison**	**Angle**	**Mean difference in m/s [95% CI]**	***P* values**
Proximal patellar tendon				
Region P<0.001 Angle P<0.001 Interaction P=0.007	Posterior vs anterior tendon	25°	-0.07 [-0.59, 0.37]	1.00
		40°	-0.16 [-0.59, 0.28]	1.00
		55°	-0.27 [-0.71, 0.16]	0.52
		70°	-0.60 [-1.03, -0.16]	<0.01
		85°	-0.97 [-1.40, -0.53]	<0.01
Middle third of patellar tendon				
Region P=0.332 Angle P<0.001 Interaction P=0.174	Posterior vs anterior tendon	25°	0.12 [-0.29, 0.54]	1.00
		40°	0.11 [-0.31, 0.53]	1.00
		55°	-0.06 [-0.48, 0.36]	1.00
		70°	-0.17 [-59, 0.25]	1.00
		85°	-0.36 [-0.78, 0.05]	0.13
Quadriceps muscle				
Muscle P<0.001 Angle P<0.001 Interaction P<0.001	Vastus lateralis vs Vastus medialis	25°	0.05 [-0.07, 0.17]	1.00
		40°	0.08 [-0.05, 0.20]	0.78
		55°	0.15 [0.03, 0.27]	<0.01
		70°	0.17 [0.05, 0.29]	<0.01
		85°	0.23 [0.11, 0.35]	<0.01
	Vastus lateralis vs Rectus femoris	25°	0.16 [0.04, 0.29]	<0.01
		40°	0.18 [0.05, 0.30]	<0.01
		55°	0.31 [0.19, 0.43]	<0.01
		70°	0.45 [0.33, 0.57]	<0.01
		85°	0.55 [0.43, 0.67]	<0.01
	Rectus femoris vs Vastus medialis	25°	-0.11 [-0.23, 0.01]	1.00
		40°	-0.10 [-0.22, -0.02]	0.21
		55°	-0.16 [-0.28, -0.04	<0.01
		70°	-0.28 [-0.40, -0.16]	<0.01
		85°	-0.32 [-0.44, -0.20]	<0.01

Pairwise comparisons with bonferroni-adjusted *P*-values are listed. Mean differences [95%] for first (compared to second) listed comparison.

Fig. 1 Elastogram of the patellar tendon with patellar (P) and tibial tuberosity (T) labelled. Regions of interest for anterior (red) and posterior (white) regions of the proximal third of the patellar tendon. Probe position, see bottom right.

Fig. 2 Elastograms for each of the quadriceps muscle regions of interest and probe positions.

Fig. 3 Shear wave velocity (mean, standard deviation) for anterior (shaded) and posterior (unshaded) regions of the proximal patellar tendon for each knee angle. Significant differences were found between all angles (not shown) and between regions as indicated by astericks.

Fig. 4 Shear wave velocity (mean, standard deviation) for the quadriceps muscles for each knee angle. Significant differences between muscles are indicated by astericks. No significant differences were found between 25° and 40° for VM and VL and between 25°, 40° and 55° for RF. All other angles were significantly different (not shown).

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