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Does epimuscular myofascial force transmission occur between the human quadriceps muscles *in vivo* during passive stretching?

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ABSTRACT

This study sought to examine the shear modulus (i.e., an force index) of three quadriceps muscles [i.e., vastus medialis (VM), vastus lateralis (VL), and rectus femoris (RF)] during passive stretching to determine whether epimuscular myofascial force transmission occurs across muscles. Secondly, this study compared the shear modulus between the quadriceps muscles, in both proximal and distal regions. Twelve healthy individuals were assessed during a passive knee flexion maneuver between 0° and 90° of knee flexion with the hip in two positions: flexed (80°) vs. neutral (0°). Muscle electrical activity was also assessed during the testing. No differences were observed between the hip testing positions for myoelectric activity ($p > 0.43$), and for VL and VM shear modulus ($p = 0.12–0.98$). Similarly, there were no differences between the proximal and distal regions for all muscles ($p = 0.42–0.93$). RF showed a higher shear modulus with the hip in the neutral position ($p = 0.004$). With the hip flexed, the VL showed the greatest shear modulus among the tested muscles ($p < 0.025$); while with the hip in the neutral position, no differences were observed for shear modulus between VL and RF ($p = 0.817$). These findings suggest that epimuscular myofascial force transmission (at a muscle belly level) does not occur between the quadriceps muscles when passively flexing the knee until 90°. Whether epimuscular myofascial force transmission occurs in the quadriceps muscles bellies with greater muscle stretch (either through knee flexion or hip extension) remains to be examined.

1. Introduction

Force transmission between skeletal muscle and its surrounding structures, also referred to as epimuscular myofascial force transmission (Huijing, 2009), has been suggested to occur during both passive stretching and muscle contraction conditions (Maas and Finni, 2017; Maas and Sandercock, 2010). However, *in vivo* investigation of epimuscular myofascial force transmission in humans is scarce. Recently, muscle shear modulus quantified by means of ultrasound-based shear wave elastography, has been shown to be strongly correlated with skeletal muscle passive force (Koo et al., 2013) and to Young's modulus (Eby et al., 2013). Therefore, muscle shear modulus can be interpreted as a passive force index. For instance, based on *ex vivo* validity experiments performed on fresh roaster chicken skeletal muscles (Koo et al., 2013), a small change in shear modulus could reflect a non-negligible alteration in muscle passive tension (e.g. change of 10 kPa ≈ 0.55–1.21 N). Recently, some studies have reported region-dependent changes in muscle shear modulus when stretching neighbouring muscles (Ateş et al., 2018; Yoshitake et al., 2018).

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For instance, the distal region of soleus from healthy human individuals was reported to exhibit a higher shear modulus during passive ankle rotations with the knee fully extended compared to a 90° knee flexion position, while the proximal soleus region showed a lower shear modulus (Ateş et al., 2018). However, whether this phenomenon is present in other muscle groups remains to be investigated.

The aponeuroses from each of the quadriceps' muscle components merge into the patellar tendon (Grob et al., 2016), and a recent investigation performed in human cadavers described a connective tissue linkage between the vastus medialis (VM) and both the rectus femoris (RF) and vastus intermedius (Grob et al., 2017). In addition, there is anatomical evidence that vastus lateralis (VL) attaches into the iliotibial band (Becker et al., 2010). Thus, it is likely that the passive force of monoarticular quadriceps components within the muscles bellies may be altered when there is a change in the length of RF or tensor fasciae latae, such as during changes in hip position. Previous studies have only compared the shear modulus of the quadriceps muscles during passive stretching with the hip in a fixed position (Coombes et al., 2018; Xu et al., 2016). In addition, contradictory findings have been reported regarding the shear modulus of the quadricep muscles under resting conditions. Coombes et al. (2018) reported that VL had the highest shear modulus of the quadriceps muscles (excluding vastus intermedius), whereas Xu et al. (2016) reported that RF had the highest shear modulus. These contradictory findings are likely to reflect the different hip position used: flexed (Coombes et al., 2018) or neutral position (Xu et al., 2016). Another relevant methodological aspect, that could possibly explain the aforementioned contradictory findings, refers to the measurement location. Le Sant et al. (2017) recently observed that the shear modulus of the medial gastrocnemius was heterogeneous, i.e., differences between proximal and distal regions were observed.

Thus, the primary aim of this study was: (i) to investigate the existence of epimuscular myofascial force transmission between the quadricep muscles during passive knee flexion (i.e., quadriceps stretching), by comparing the shear modulus within the VM and VL muscle bellies with the hip flexors at two lengths by changing the hip position: hip flexed (80°) vs. hip neutral (0°) positions. Secondly, we aimed to compare the shear modulus (ii) between and (iii) within (i.e., proximal vs. distal regions) the quadricep muscles (VM, VL, RF), with the hip flexors at different lengths. We hypothesized that: (i) both the VM and VL would present a higher shear modulus when hip flexors assumed a lengthened condition, due to the potential epimuscular myofascial force transmission; (ii) VL would show the highest shear modulus with the hip flexed, while the RF would show the highest shear modulus with the hip in neutral position; and (iii) distal muscle regions would show greater shear modulus due to its proximity to the joint being mobilized.

2. Methods

2.1. Participants

Twelve healthy and recreationally active male individuals (age: 23.7 ± 3.6 yrs; height: 1.76 ± 0.08 m; body mass: 71.2 ± 8.9 kg) volunteered for this study. Participants provided written informed consent, and reported no history of neuromuscular injuries in the lower limbs. Sample size was estimated using the G*Power software (v3.1.9.2, Düsseldorf, Germany), for a test-retest reliability outcome of 0.75, effect size of 0.2, statistical power of 80% and significance of 5%. This study was approved by the local Ethics committee.

2.2. Equipment and variables

2.2.1. Dynamometry

An isokinetic dynamometer (Biodex System 3 research, Shirley, NY, USA) was used to impose knee flexion passive stretching at 1°/s, and to assess the knee angle at 1000 Hz during the stretching trials. One limb (randomly chosen) was tested. The dynamometer axis was aligned with the lateral femur condyle. Knee flexion occurred between 0° and 90°. Care was taken to position the knee back to full extension when 90° of knee flexion was achieved in order to prevent quadriceps stress relaxation, as it was previously shown in medial gastrocnemius (by means of shear modulus decrease) when performing passive stretching (Freitas et al., 2015). Participants assumed two positions throughout the protocol, starting in a random order: seated with the hips at 80°; and lying supine with the hips at 0°. These positions placed the RF and tensor fasciae latae in a shorter and longer length, respectively.

2.2.2. Shear wave elastography

An ultrasound scanner (Aixplorer, v11; Supersonic Imagine, Aix-en-Provence, France) defined in shear wave elastography setting (musculoskeletal preset, penetrate mode, smoothing level 5, persistence off; scale: 0–200 kPa), coupled with a linear transducer array (4–15 MHz. Super Linear 15-4, Vermon, Tours, France) was used to assess the muscle shear modulus of VL, VM, and RF in both proximal and distal regions. Vastus intermedius was not assessed due to difficulties in obtaining repeatable elastograms within the same session. Care was taken to apply minimal transducer pressure during the shear wave measurements, in order to minimize the methodological error (Kot et al., 2012). One video clip was recorded for each muscle. The elastogram push frequency was automatically set by the ultrasound scanner to ~1 Hz (range: 0.8–1.4 Hz). To ensure a stable and repeatable transducer positioning, a custom-made plastic cast was fixed to the skin over each target region of interest at ~20–40% and ~60–80% of each muscle's proximal-to-distal length, according to the fascicle orientation and without visualizing other structures than fascicles, like vessels or intramuscular aponeurosis (Fig. 1A).

2.2.3. Surface EMG

A telemetric EMG system (Plux, Lisbon, Portugal) was used to assess the muscle electrical activity during the stretch testing. A pair of EMG electrodes (bipolar Al/AgCl electrodes with an inter-electrode center-to-center 20-mm distance; AMBU, Ballerup, Denmark) were placed at ~50% of each muscle proximal-to-distal length, according to the fascicles orientation, through guidance of ultrasonography (Fig. 1A). EMG signals were acquired at 1000 Hz.

2.3. Protocol

The participants were tested in one session. At the participant's arrival, the skin of the thigh was prepared for placing the EMG electrodes and the casts to place the ultrasound probe (Fig. 1A). Prior to the testing protocol, five knee flexion-extension (between 0 and 90°) cycles were performed at 5°/s for conditioning purposes. Participants were instructed to fully relax during the stretching. The protocol consisted of 12 stretching trials for each hip position with 1-min rest between trials, as two repetitions were performed for each muscle region (i.e., 3 muscle × 2 regions × 2 repetitions). At the end of the protocol, two 4-s maximal knee extension isometric contractions were performed (with the knee and hip fixed at 80° flexion) in order to normalize EMG signals. Signal synchronization was ensured using an external trigger that activated the start of data acquisition for the elastography videoclips, dynamometry data, and electromyographic data.

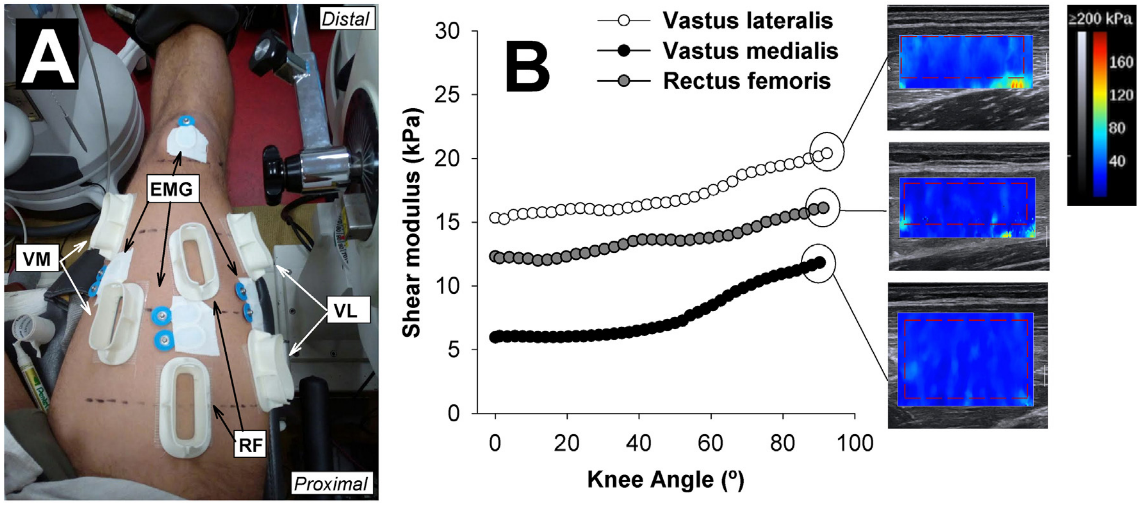

Fig. 1. (A) Typical ultrasound transducer casts and electromyographic electrode positioning; and (B) example of a raw shear modulus-knee angle curve of the distal region of vastus medialis, vastus lateralis, and rectus femoris from one participant (#6), with the hip flexed at 80°. Dashed red rectangles over the elastograms represent the region of interest to determine the muscle shear modulus. The information displayed in the elastogram windows (and in the coloured scale) indicate Young modulus values, while the y-axis of the graphs presents the muscle shear modulus (i.e. Young modulus divided by 3). VM – vastus medialis; VL – vastus lateralis; RF – rectus femoris.

2.4. Data processing

All data was processed using custom-made Matlab scripts (The Mathworks Inc., Natick, Massachusetts, USA). For shear modulus calculation, only pixels of the color map containing the muscular region of interest were selected by manually avoiding aponeurosis areas surrounding the muscle bellies or other non-muscular structures (which could affect muscle shear modulus measurements), and automatically removing missing value pixels. The mean pixel value was converted to a shear modulus value based on the recorded color scale in kPa, and by dividing Young's modulus by three to best approximate the muscle shear modulus (Bercoff et al., 2004). EMG signals were band-pass filtered (20–500 Hz), smoothed by determining the root mean square (RMS) over consecutive moving windows of 250 ms, and normalized to the highest EMG activity observed during maximal contraction. Using a linear interpolation technique, the % of EMG activity and the shear modulus of each muscle was determined for each 20% over the tested 0–90° of knee flexion (i.e. each 18°). The average of the two repetitions for each muscular region assessment was analysed.

2.5. Statistical analysis

Analysis was performed using SPSS software (version 22.0, IBM, Chicago). Data normality was confirmed using the Shapiro-Wilk test. The shear modulus measurement repeatability was checked for each muscle by calculating the intraclass correlation coefficient ($ICC_{3,1}$), and classified as little ($ICC < 0.25$), low (0.26–0.49), moderate (0.50–0.69), high (0.70–0.89), and very high (>0.90) (Domholdt, 1993). A two-way repeated measures ANOVA [within-subject factors: hip positions (flexed, neutral) × knee 0–90° ROM percentiles (20, 40, 60, 80, 100)] was performed to compare the EMG activity of each muscle (VL, VM, RF) between hip positions along the 0–90° of knee flexion. A three-way repeated measures ANOVA [within-subject factors: hip positions (flexed, neutral) × regions (distal, proximal) × knee 0–90° ROM percentiles (20, 40, 60, 80, 100)] was performed to compare the shear modulus of each muscle (VL, VM, RF) along the 0–90° of knee flexion between the two hip positions. A three-way repeated measures ANOVA [within-subject factors: muscles (VL, VM, RF) × regions (distal, proximal) × knee 0–90° ROM percentiles (20, 40, 60, 80, 100)] was performed for each tested hip position to examine shear modulus differences between muscles. When a main effect was found, post-hoc Bonferroni tests were performed. Significance was considered for $p < 0.05$.

3. Results

A typical shear modulus response for the tested muscles can be observed in Fig. 1B. A high to very high shear modulus measurement repeatability was found across muscles (RF: $ICC_{3,1} = 0.73$–0.99; VL: $ICC_{3,1} = 0.77$–0.98; VM: $ICC_{3,1} = 0.74$–0.99). A very low muscle electrical activity was observed during the stretching maneuvers (VL = 0.3–0.5%, RF = 0.4–0.5%, VM = 0.7–1.3%), with no significant difference between hip positions ($p = 0.25$–0.43) nor knee ROM percentiles ($p = 0.22$–0.31); suggesting that testing was performed in a passive condition.

The shear modulus of all muscle regions at given percentages of knee ROM are shown in Fig. 2. For VL and VM, a main effect was observed for knee ROM percentiles ($p < 0.001$); but there were no main effects of hip position ($p = 0.18$–0.90) or muscle regions ($p = 0.42$–0.93), and no interactions were observed between the factors ($p > 0.08$). For RF, a main effect was found for the hip position ($p = 0.004$) and knee ROM percentiles ($p < 0.001$), as well as a hip position × knee ROM percentiles interaction ($p = 0.008$); while no effect was found for muscle regions ($p = 0.589$) or other type of interactions between factors ($p > 0.07$).

Regarding the comparison between muscles (Fig. 3), for the hip flexed position a main effect was found for muscle ($p = 0.002$), knee ROM percentiles ($p < 0.001$), and muscle × knee ROM percentiles interaction ($p = 0.007$). No effect was seen for regions ($p = 0.49$), muscle × regions ($p = 0.959$), regions × knee ROM percentiles interaction ($p = 0.103$), nor muscle × regions × knee ROM percentiles interaction ($p = 0.531$). Post hoc comparisons revealed that VL was stiffer than VM ($p = 0.004$) and RF ($p = 0.025$), and no differences were found between VM and RF ($p = 1.00$). For the hip neutral position (Fig. 3), a main effect was found for muscle ($p < 0.001$), knee ROM percentiles ($p < 0.001$), and muscle × knee ROM percentiles interaction ($p = 0.004$). No effect was seen for regions ($p = 0.757$), muscle × regions ($p = 0.741$), regions × knee ROM percentiles interaction ($p = 0.07$), nor muscle × regions × knee ROM percentiles interaction ($p = 0.062$). Post-hoc comparisons revealed

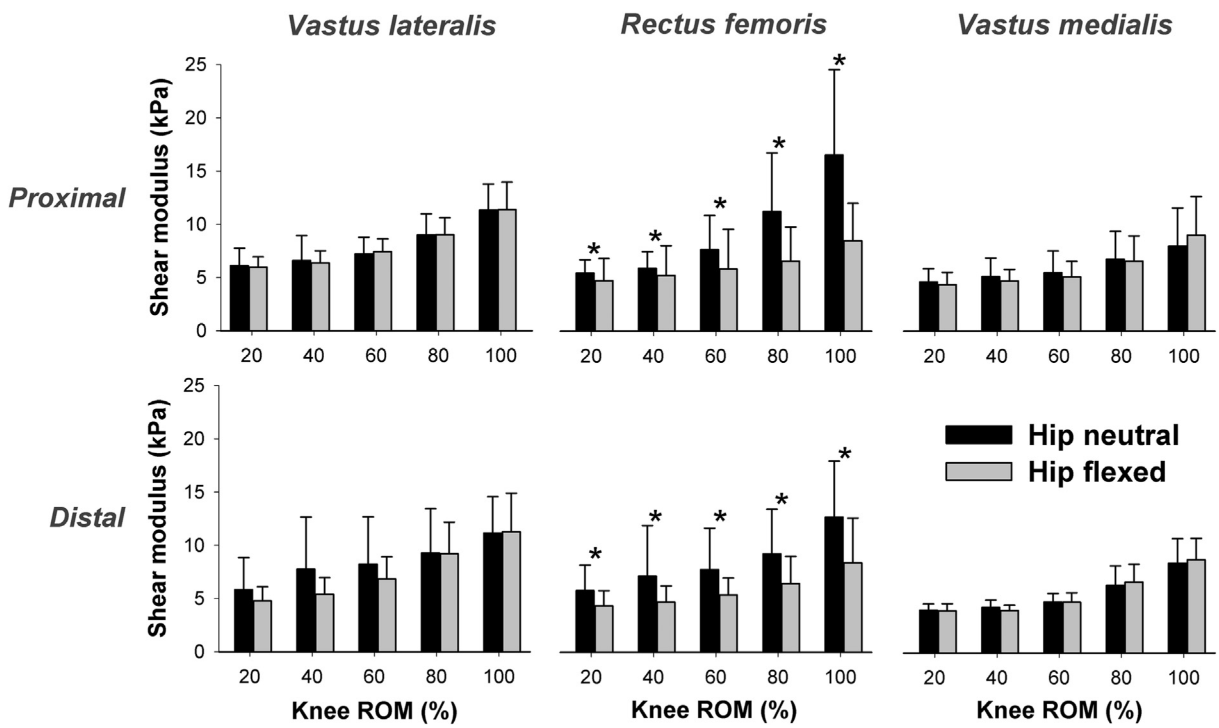

Fig. 2. Shear modulus for each knee range of motion percentile of the proximal and distal regions of vastus medialis, rectus femoris, and vastus lateralis in two hip positions: neutral (0°) and flexed at 80°. Mean ± SD values are plotted. * Indicates statistical differences between hip positions ($p < 0.05$).

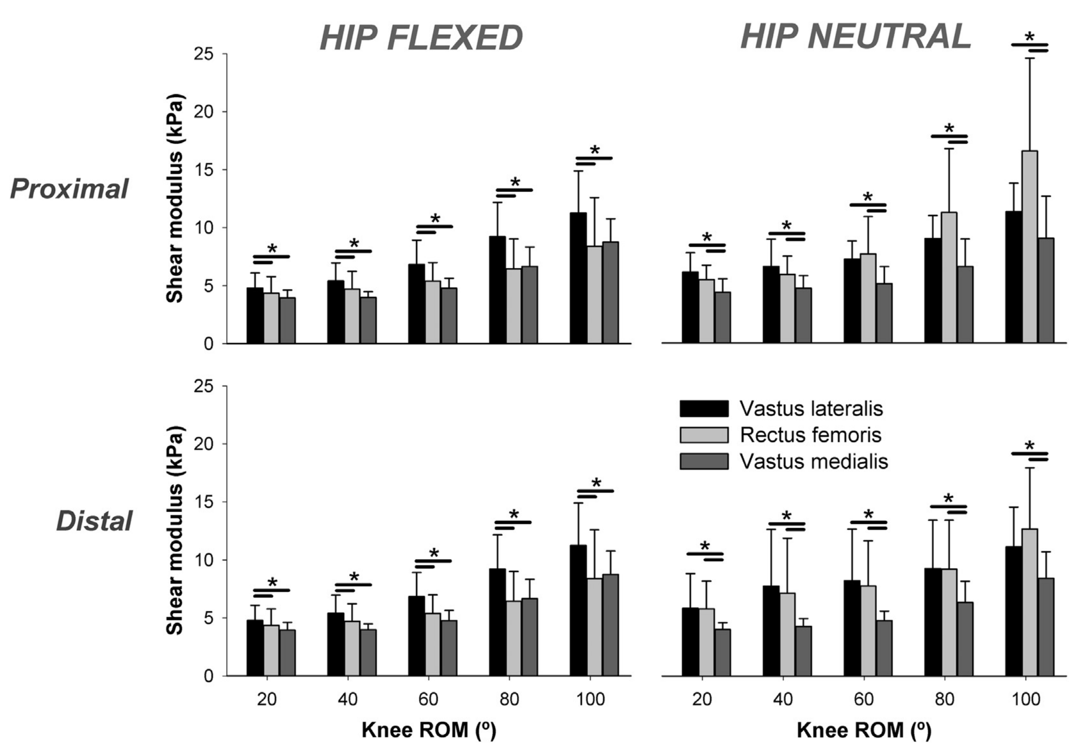

Fig. 3. Shear modulus-range of motion curves for the proximal and distal regions of vastus medialis, rectus femoris, and vastus lateralis in either hip neutral (0°) or flexed (80°) positions. Mean ± SD values are plotted. Data shown in this figure is the same as for Fig. 2, but differently organized to provide a better, and different, readability. * Indicates statistical differences between muscles ($p < 0.05$).

that VL was stiffer than VM ($p = 0.001$), RF was stiffer than VM ($p = 0.001$), and no differences were found between VL and RF ($p = 0.817$).

4. Discussion

This study investigated the existence of epimuscular myofascial force transmission between the quadriceps muscles during passive knee flexion. We compared VM and VL shear modulus with the hip flexors at two lengths by manipulating the hip position: flexed (80°) and neutral (0°). The present study results contradict our hypothesis that both VM and VL would present a higher shear modulus with the hip in a neutral position (i.e., hip flexors more lengthened). Instead, we observed no differences between hip positions in VM and VL shear modulus. This study also compared the shear modulus between and within the quadriceps muscles in the same two hip positions. Our hypothesis, that VL would exhibit the highest shear modulus with the hip flexed and RF with the hip in a neutral position, was partially confirmed. We observed VL had the highest shear modulus amongst the quadriceps muscles with the hip flexed, while no shear modulus differences between RF and VL were found with the hip in a neutral position. Lastly, we also hypothesized that distal muscle regions would show greater shear modulus due to its proximity to the joint being mobilized; but no differences were found between regions.

Considering the (i) connective tissue-based linkages between the RF and VM (Grob et al., 2017), and between VL and the iliotibial band (Becker et al., 2010), as well as (ii) the evidence regarding passive force transmission among synergistic muscles (Ateş et al., 2018; Yoshitake et al., 2018), we expected a shear modulus change in VM and VL between the hip positions. Please note that a small change in muscle shear modulus reflects a non-negligible change in muscle passive tension (Koo et al., 2013). Based on the present results, we admit three possibilities. First, the changes in force may only occur at a connective tissue level surrounding the muscles (e.g. aponeurosis), and do not achieve the muscle bellies. Interestingly, a recent study reported that the fascicles length of soleus from Wistar rats did not changed when the gastrocnemius and plantaris muscle-tendon units length was increased, but it was noted a considerable high increase of force at the tendon level from the soleus (Tijs et al., 2018). Important to note that (fascicles) deformation measurements do not represent a direct force measurement. Nevertheless, it may be possible that epimuscular myofascial force transmission occur at a connective tissue level, and only achieve the muscle bellies with higher levels of (intermuscular) connective tissue strain. It should be noted that the hip position varied between neutral (0°) and 80° flexion, and the knee was only flexed up to 90°. Such positions are within the physiological joint range of motion performed in tasks of daily living (e.g., seated vs. upright positions). Thus, the stretch that both the RF and the iliotibial band went through may not have been enough to produce a passive force transmission to monoarticular quadriceps muscles. A second possibility is that force transmission among the quadriceps muscles during passive stretching in healthy people is negligible, regardless of the degree of muscle lengthening. This may not be the case for some clinical populations; for instance, in people with chronic stiff arthritic knee (Tarabichi and Tarabichi, 2010), as they present considerable joint range of motion deficits. Finally, we cannot exclude the possibility that there is epimuscular myofascial force transmission between the quadriceps closer to the patellar tendon, as the aponeurosis from the quadricep muscle components' merge onto the patellar tendon (Grob et al., 2017, 2016). Although we found no differences between proximal and distal muscle regions, note that the distal shear modulus measurements were performed at ~60–80% of each quadricep muscles' proximal-to-distal length.

Similar to the findings of Coombes et al. (2018) observed, we showed that the shear modulus of VL was the highest among the quadriceps components during passive stretching when the hip was flexed, although we used a more flexed hip position (80° vs. 60°). On the other hand, and contrary to Xu et al. (2016) observations (which reported a higher RF shear modulus, and similar shear modulus values between VL and VM), and contrary to our initial hypothesis, a similar shear modulus was observed between RF and VL when the hip was in a neutral position, and a greater shear modulus was found in VL compared to VM. Note that the hip was slightly flexed (i.e., 10°) from the hip neutral position in the Xu et al. (2016) study, which would place RF in a slightly shorter length compared to our study. Although the RF stretching caused by the more extended hip position increased the RF shear modulus, it was insufficient to be statistically higher than VL values. We are uncertain of the reason that explains such differences. A possible explanation is that the rowing individuals tested by Xu et al. (2016) may present a specific shear modulus distribution within the quadriceps muscles compared to our non-athletic population. Thus, the effect of training could be an interesting topic for future research.

Based on previous studies that showed a greater shear modulus value in the muscle region closer to the joint where the passive stretching was performed (Le Sant et al., 2017), and a higher muscle tension closer to site of the tissue that was being stretched (Huijing and Baan, 2001), we expected a higher muscle shear modulus in the distal regions of the quadriceps muscles during the passive stretching imposed by knee flexion. However, we did not observe an effect for the muscle region factor, which suggests that the shear modulus response during passive stretching is somewhat similar between proximal and distal regions of the quadriceps muscles. It should be noted that knee flexion was not performed until the maximal ROM; thus, we do not know if a similar conclusion would be obtained with greater quadriceps extensibility.

In conclusion, the present study showed *in vivo* that the shear modulus of the vastus lateralis and vastus medialis of healthy individuals remained unchanged between two hip positions during passive knee flexion up to 90°. A future study should examine if the epimuscular myofascial force transmission during a passive stretching condition mainly occurs at a connective tissue level, and only occur within the muscle belly for higher levels of myofascial stretch (e.g., by evoking greater knee flexion). In addition, the shear modulus of vastus lateralis was the greatest with the hip flexed, while both vastus lateralis and rectus femoris showed the highest shear modulus when the hip was in the neutral position. No shear modulus differences were seen between proximal and distal regions. As the stiffness differences between synergistic muscle may differ depending the type populations (e.g., trained athletes, or clinical populations), this issue should be examined in a future study.

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Conflict of interest statement

The authors disclose no conflict of interest.

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