Original article

Stiffness of individual quadriceps muscle assessed using ultrasound shear wave elastography during passive stretching

Jingfei Xu [a,b,c], François Hug [d,e], Siu Ngor Fu [b,*]

[a] Department of Rehabilitation Medicine, West China Hospital, Sichuan University, Chengdu 610041, China
[b] Department of Rehabilitation Sciences, The Hong Kong Polytechnic University, Hong Kong, China
[c] Institute for Disaster Management and Reconstruction, Sichuan University, Chengdu 610207, China
[d] The University of Queensland, NHMRC Centre of Clinical Research Excellence in Spinal Pain, Injury and Health, School of Health and Rehabilitation Sciences, Brisbane 4072, Australia
[e] Laboratory "Movement, Interactions, Performance" (EA 4334), University of Nantes, Nantes 44000, France

Abstract

Background: Until recently it has not been possible to isolate the mechanical behavior of individual muscles during passive stretching. Muscle shear modulus (an index of muscle stiffness) measured using ultrasound shear wave elastography can be used to estimate changes in stiffness of an individual muscle. The aims of the present study were (1) to determine the shear modulus−knee angle relationship and the slack angle of the vastus medialis oblique (VMO), rectus femoris (RF), and vastus lateralis (VL) muscles; (2) to determine whether this differs between the muscles.
Methods: Nine male rowers took part in the study. The shear modulus of VMO, RF, and VL muscles was measured while the quadriceps was passively stretched at $3°/s$. The relationship between the muscle shear modulus and knee angle was plotted as shear modulus−knee angle curve through which the slack angle of each muscle was determined.
Results: The shear modulus of RF was higher than that of VMO and VL when the muscles were stretched over $54°$ (all $p < 0.01$). No significant difference was found between the VMO and VL (all $p > 0.05$). The slack angle was similar among the muscles: $41.3° \pm 10.6°$, $44.3° \pm 9.1°$, and $44.3° \pm 5.6°$ of knee flexion for VMO, RF, and VL, respectively ($p = 0.626$).
Conclusion: This is the first study to experimentally determine the muscle mechanical behavior of individual heads of the quadriceps during passive stretching. Different pattern of passive tension was observed between mono- and bi-articular muscles. Further research is needed to determine whether changes in muscle stiffness are muscle-specific in pathological conditions or after interventions such as stretching protocols.
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Keywords: Muscle tension; Optimal length; Shear modulus; Slack angle; Stretch; Ultrasonography; Vastus lateralis; Vastus medialis

1. Introduction

Flexibility is classically assessed at the joint level by measuring the maximum range of motion or the joint torque during passive motion.[1] However, these measures are influenced by the contribution of many structures crossing the joint including muscles, nerves, and skin. Hence, the behavior of individual muscles is not directly represented. This is problematic as there is recent evidence that stretching-induced change in muscle stiffness may differ between individual muscles that belong to the same muscle group such as hamstring muscles.[2]

Therefore, it is important to assess each individual muscle for a deeper understanding of muscle flexibility and to improve musculoskeletal models.

Ultrasound shear wave elastography is a technique to quantify the stiffness of a localized area of soft tissue. An elastography technique called supersonic shear imaging (SSI) provides an accurate quantification of muscle shear modulus[3] that can be considered as a measure of muscle stiffness.[4] Because there exists a strong linear relationship between muscle stiffness and passive tension when passively stretching the muscle,[5] changes in muscle stiffness measured using SSI can be used to estimate tension changes of muscle responding to passive stretch.[6] In addition, the SSI method provides an opportunity to estimate the slack angle of individual muscles which is

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Peer review under responsibility of Shanghai University of Sport.
* Corresponding author.
  E-mail address: amy.fu@polyu.edu.hk (S.N. Fu).

defined as the joint angle beyond which muscles begin to develop passive tension.[7]

Taking advantage of SSI, changes in muscle stiffness have been estimated during passive stretching in humans, mainly on the medial gastrocnemius,[7−9] soleus,[8] tibialis anterior,[10] and biceps brachii muscle.[11] All these studies confirmed the classic exponential relationship between passive muscle tension and muscle length. Interestingly, different stiffness values were reported between muscles belonging to the same muscle group such as hamstring muscles.[2] Further, Hirata et al.[8] demonstrated that the individual heads of the triceps surae exhibit a different slack angle during passive dorsiflexion with larger plantar flexed angle for the medial gastrocnemius than the lateral gastrocnemius and the soleus. In contrast, both heads of the biceps brachii muscle have the same slack angle at about 95° elbow flexion.[11]

It is important to assess the passive behavior of the quadriceps muscle heads from both a clinical and basic sciences perspective. First, the quadriceps muscle is a large muscle group that is exposed to large strain while individuals perform pushing/pulling movements and running or jumping actions.[12] As a consequence, along with the hamstrings and triceps surae, quadriceps is one of the 3 muscle groups that are the most susceptible to be injured in athletes.[13] The strain injuries commonly occur in the rectus femoris (RF) muscle,[14] suggesting that the stiffness of the RF muscle is higher than that of the other mono-articular heads. Second, although biomechanical models often consider that the slack angle of muscle is similar to the optimal angle at which the maximal force can be generated,[10,15] there exists no experimental evidence of this assumption. SSI provides a unique opportunity to test this assumption.

We designed this study (1) to determine the passive stiffness−angle relationship and the slack angle of the vastus medialis oblique (VMO), RF, and vastus lateralis (VL) muscles; (2) to determine whether this differs between muscles. Muscle shear modulus (an index of stiffness) was measured using SSI during passive knee flexions. The vastus intermedius (VI) muscle was not recorded because its location underneath VM, VL, and RF made challenging to get reliable measurements.

2. Materials and methods

2.1. Participants

Nine male rowers without history of leg injury (age: 21.4 ± 2.2 years, height: 177.2 ± 5.1 cm, body mass: 67.5 ± 5.5 kg) participated in this study. All the participants volunteered for this study and provided informed written consent. This study has been approved by the local Human Subject Ethics Subcommittee (Department of Rehabilitation Sciences, The Hong Kong Polytechnic University).

2.2. Passive stretching

An isokinetic dynamometer (Cybex, Medway, MA, USA) was used to impose passive knee flexions. Before the participant was positioned on the dynamometer, the location of the ultrasound transducer was determined on the skin using a waterproof pen for each muscle (VMO: 20% of the distance from the midpoint of medial patella border to anterior superior

iliac spine as the test position; RF: 50% of the distance from anterior superior iliac spine to the midpoint of the superior tip of the patella; VL: 1/3 of the distance from the midpoint of lateral patella border to anterior superior iliac spine). Then, participants were positioned supine with their hip flexed at 10° using a customized cushion put on the dynamometer bed to avoid hip hyperextension. The dominant leg, determined by the ball kicking test, was measured. The hip was positioned in a neutral position and the presumed axis of the knee rotation was aligned with the axis of the dynamometer.

2.3. Muscle shear modulus measurements

An Aixplorer ultrasound scanner (Aixplorer Version 4.2; Supersonic Imagine, Aix-en-Provence, France), coupled with a linear transducer (4−15 MHz, Super Linear 15-4; Supersonic Imagine) was used in shear wave elastography mode (MSK preset) to measure the Young's modulus assuming isotropic nature of soft tissues. As skeletal muscle cannot be assumed to be isotropic, we reported the shear modulus values as the Young's modulus values divided by 3.[6]

An experienced examiner performed all the measurements. The transducer was first oriented in the transverse plane to ensure that the right muscle was measured and then rotated to be parallel to the muscle fascicle direction. For VMO and VL, the optimal transducer location was determined when several muscle fascicles could be seen without disconnection through the image.[12] Because of the complex arrangements of RF fascicles, the transducer was placed over the lateral component of this muscle[16−18] and oriented in muscle shortening direction.

The 2-dimensional (2D) maps of muscle shear modulus were captured for 1 sample with a spatial resolution of 1×1 mm. Although the size of region of interest (ROI) does not have a significant impact on the shear modulus,[19] the ROI was set as big as possible according to the muscle thickness (about 2.25 cm^2 for VMO and VL, $1.5−2.5$ cm^2 for RF) to achieve accurate value of passive tension.

2.4. Experimental protocol

After a 10 min rest period, the participant's quadriceps muscle was passively stretched through slow loading cycles (3°/s) from 0° (full knee extension) to 120° of knee flexion. Before the start of loading cycle, a 5 s rest period was used to optimize the position of the transducer. A self-customized trigger was used to synchronize the Cybex dynamometer and SSI scanner, that is, to start the elastography measurements at the start of the loading cycle. Oral instruction was given to the participants to stay relaxed and avoid any muscle contraction and movement of the leg throughout the passive stretching. The test sequences of the 3 muscles were randomly arranged.

2.5. Data analysis

SSI data were exported in .mp4 format and sequenced in .png format. Image processing was performed using a custom Matlab script (MathWorks, Natick, MA, USA). Each image was carefully inspected for artifacts. If artifacts were present in any image, the ROI was reduced in size to remove
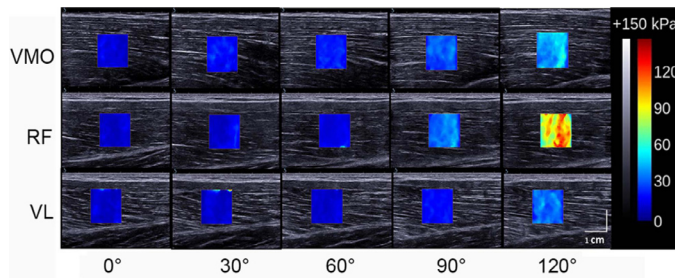
Fig. 1. Typical example of maps of shear modulus obtained during passive knee flexion at 0°, 30°, 60°, 90°, and 120°. The map of shear modulus is superposed onto a B-mode image, with the color scale depicting graduation of shear modulus. To obtain a representative value, the shear modulus was averaged over the region of interest. RF = rectus femoris; VL = vastus lateralis; VMO = vastus medialis oblique.
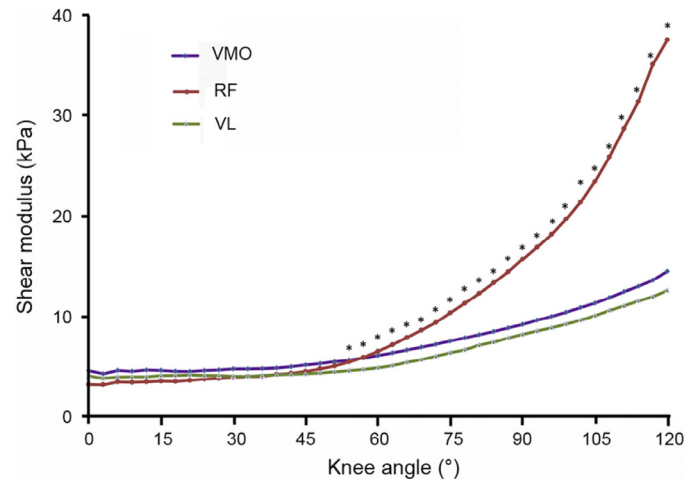

Fig. 2. Averaged passive shear modulus−knee angle curve obtained during passive knee flexion. The standard deviation error bars are not included for clarity. The shear modulus was higher for rectus femoris (RF) than vastus medialis oblique (VMO) and vastus lateralis (VL) when the knee was passively stretched above 54° (*$p < 0.01$). No significant difference was found between VMO and VL.

the artifact from all images within that stretching condition. The colored 2D maps of shear modulus were converted into shear modulus values and the shear modulus was averaged over the ROI on each image. Then, the shear modulus−knee angle relationship was plotted and the slack angle was visually determined by an experienced examiner.[7,11] The slack angle determined by this method has been shown to be similar to that calculated from the exponential model[7,9] and good intra-session and inter-rater reliability of this visual determination has been reported.[7,8]

2.6. Statistical analysis

Statistical analysis was conducted using SPSS software package for Windows (Version 21.0; IBM Corp., Armonk, NY, USA). Data distribution consistently passed the Shapiro−Wilk normality test. One-way repeated measures ANOVA (within-subject factor: muscle) was used to compare the slack angle between the 3 muscles. A two-way repeated measures ANOVA (within-subject factors: muscle and knee angle) was used to compare the shear modulus values. *Post hoc* analyses were performed when appropriated using the Bonferroni method. Statistical significance was set at $p < 0.05$.

3. Results

Representative maps of muscle shear modulus are depicted in Fig. 1. There was a main effect of "knee angle"

($p < 0.0001$) showing that the shear modulus increased for each muscle while the muscle was passively stretched (Fig. 2), reaching a maximum at the end of joint movement (120° of knee flexion in the present study). There was a significant interaction between the factors "muscle" and "knee angle" ($p < 0.0001$). The shear modulus was higher for RF than VMO and VL when the knee angle was stretched above 54° (all $p < 0.01$). No significant difference was found between the VMO and VL in the whole range of motion (all $p > 0.05$). The maximal shear modulus of RF measured at 120° of knee flexion was significantly higher (37.5 ± 8.8 kPa) than that measured for VMO (14.5 ± 1.8 kPa; $p = 0.0001$) and VL (12.6 ± 2.6 kPa; $p = 0.00003$).

Fig. 3 depicts a typical example of visual determination of the slack angle from shear modulus−knee angle curve of VMO, RF, and VL. The slack angle was not different between the muscles (main effect of muscle $p = 0.626$) with values of $41.3° \pm 10.6°$ (95%CI: $33.2° − 49.5°$), $44.3° \pm 9.1°$ (95%CI: $37.3° − 51.3°$), and $44.3° \pm 5.6°$ (95%CI: $40.1° − 48.6°$) for VMO, RF, and VL, respectively.
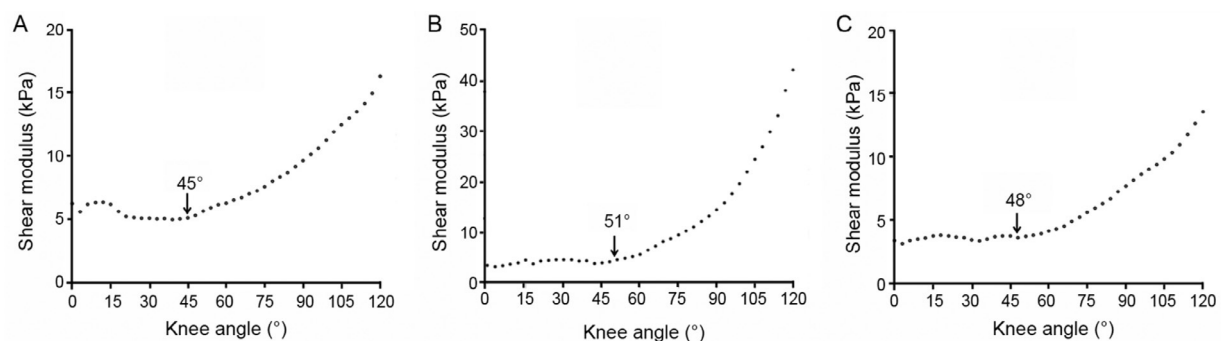

Fig. 3. Typical example of visual determination of slack angle from the shear modulus−knee angle curve of (A) vastus medialis oblique (VMO), (B) rectus femoris (RF), and (C) vastus lateralis (VL). The arrows show the angle above which the passive muscle tension begins to increase.

4. Discussion

The aim of the present study is to take advantage of SSI to determine the passive shear modulus−knee angle relationship and the slack angle of each of the 3 superficial heads of the quadriceps muscle. The shear modulus of RF was higher than that of VMO and VL above 54° of knee flexion. We found no difference in slack angle between the 3 muscles.

To our knowledge, this is the first report to investigate the shear modulus−knee angle relationship of individual quadriceps muscle during passive knee stretching. As expected, the VMO, RF, and VL exhibited a similar behavior, i.e., an exponential increase in passive tension after the muscles were stretched over the slack angle with a maximum tension value reached at the end of motion. No significant difference was found in shear modulus between VMO and VL over the entire range of knee flexion. This is consistent with finding reported by Bensamoun et al.[20] who measured the shear modulus of VMO and VL at 30° of knee flexion using magnetic resonance elastography (MRE). The present study provides further evidence to expand this earlier observation from 1 knee angle to almost the whole range of motion (0° to 120° knee flexion). In contrast, shear modulus of RF increased more sharply than VMO and VL exhibiting a steeper shear modulus−knee angle curve above 54° of knee flexion. It is important to consider methodological explanations for this higher RF stiffness. The ultrasound transducer was oriented in muscle shortening direction for RF. However, because of muscle anisotropy the shear modulus is highest when the ultrasound transducer is parallel to the muscle fibres.[21] As such, the fact that the ultrasound transducer was not aligned with muscle fascicles for RF meant that the ultrasound transducer was likely to underestimate the shear modulus values rather than to overestimate it. We therefore believe that our conclusion, that RF modulus is higher than VL and VMO, is still valid. This higher stiffness may be explained by the function of this muscle that is involved in both knee extension and hip flexion. In this way, the extended position of the hip adopted by the participants in the present study induced additional stretching of RF. Since a relatively steep passive tension−length curve indicates a fairly stiff muscle,[22] the sharply increasing shear modulus of RF demonstrates the high stiff property of this muscle, which might be related to a lower flexibility and higher risk of muscle injuries.[1,23] The present results are in accordance with the clinical observation that the much higher prevalence of muscle strain occurred in RF than vasti muscles.[14]

Although RF exhibited a higher passive stiffness than VL and VMO above 54° of knee flexion, the slack angle was similar across the 3 investigated muscles (about 40° of knee flexion). The similarity in slack angle among the 3 superficial heads of quadriceps muscle indicates that they behave as a whole at the onset of responding to the passive stretching. A comparable slack angle among synergist muscles was also reported in biceps branchii muscle.[11] In contrast, Hirata et al.[8] reported a disparity in slack angle among the triceps surae muscle group. Taken together, these results highlight the need to experimentally determine the slack angle of individual muscles even in the same muscle group.

Biomechanical models often consider that the slack angle of muscle is similar to the optimal angle at which the maximal force can be generated.[10,15] As the optimal angle for the knee extensor muscle has been reported to be 70°−80° of knee flexion,[24-26] the present results provide strong experimental evidence that there is a substantial difference between slack angle and optimal angle for this muscle group. In addition, it is important to consider that the participants were in a supine position in the present study while the optimal angle was often determined when the participants were seated. Due to the lower stretch of RF in seated position compared to the supine position, it is possible that the knee needs to be more flexed so that the quadriceps muscle group can produce maximal force. Interestingly, our results highlight an inter-individual variability of the slack angle (95%CI: 33°−51°). This result suggests that it is important to experimentally determine the slack angle of each individual muscle when this parameter has to be used in musculoskeletal model, and the SSI is a unique technique to make this determination possible.

The present study requires consideration of 2 limitations. First, myoelectrical activity was not recorded to ensure that the muscles remained passive. However, similar to what was done in previous studies,[10,11] the participant was verbally instructed to stay relaxed before each knee flexion. In addition, a researcher monitored the B-mode images during each measurement. If any muscle contraction was detected on the B-mode images, the data were discarded and another measurement was performed. Second, the participants were in a supine position to ensure that the hip was extended, as this is the case during the activities where RF strains are caused. Therefore, it should be prudent to extrapolate the results to other hip positions.

5. Conclusion

This is the first study to experimentally determine the mechanical muscle behavior of individual heads of the quadriceps during passive stretching using ultrasound shear wave elastography. The present results show that the superficial heads of quadriceps muscle start to generate passive tension almost at the same knee angle (slightly over 40° flexion) during passive knee flexion. Different pattern of passive tension was observed between mono- and bi-articular muscles. Further research is needed to determine whether changes in muscle stiffness are muscle-specific in pathological conditions or after interventions such as stretching protocols.

Authors' contributions

JX carried out the study concepts and design, data collection, statistical analysis, manuscript preparation; FH participated

in the statistical analysis and helped to draft the manuscript; SNF conceived of the study, participated in coordination, and helped to edit the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

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