DIVERGENCE ON MUSCLE STIFFNESS OF INDIVIDUAL MUSCLE HEADS OF THE QUADRICEPS FEMORIS MUSCLE

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XU JINGFEI

A thesis submitted in partial fulfillment of the requirements
for the Degree of Doctor of Philosophy

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CERTIFICATE OF ORIGINALITY

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ABSTRACT

The quadriceps femoris muscle relies on the synchronized action of its four muscle heads, controls the position and movement of the patella–femoral and tibio–femoral knee joints. Dissimilarity of structure and function have been reported on individual heads of the quadriceps femoris muscle. Divergence on their mechanical properties might cause mal-alignments of the patella–femoral and tibio–femoral knee joints and might affect muscle function and performance.

Hence, the overall objective of the study was to investigate whether modulation on mechanical properties of quadriceps femoris muscle would be muscle-head specific. In order to address this objective, four inter-related studies were conducted. The superficial muscle heads of interest were the vastus medialis (VM), rectus femoris (RF), and vastus lateralis (VL) muscles.

Muscle shear modulus (an index of stiffness) was measured using shear wave elastography. Study 1 aimed to determine the passive stiffness–angle relationship and the slack angle of the three muscle heads and to explore whether this differs between muscle heads. Study 2 aimed to study aging effects by comparing the (1) passive shear modulus of the three muscle heads at 30º, 60º, and 90º of knee flexion and (2) active shear modulus of these muscle heads during isometric contraction (30% of maximal isometric voluntary contraction (MIVC)) at 30º, 60º and 90º of knee flexion between 20 healthy young and 20 senior females. Study 3 had a similar study design as study 2 and comparison on the outcome measures were conducted between 20 healthy females and 10 males. The effect of eccentric exercise on passive muscle stiffness was explored in study 4. Whether a bout of eccentric exercise would change the passive stiffness of individual heads of quadriceps femoris muscle homogenously was determined and whether pre-exercise muscle stiffness is related to the force loss observed after a bout of eccentric knee

extensions at immediately post- and 48 hours post-exercise on 50 young healthy subjects was explored.

Results from the 4 studies indicate a divergence in response to joint positioning, aging, gender and post eccentric exercises modulation on passive muscle stiffness among the muscle heads of the quadriceps femoris muscles. The study showed that the shear modulus of RF muscle was higher than that of VM and VL when the muscles were stretched over 54°. No significant difference was found between the VM and VL. The slack angle was similar among the muscle heads which was just over 40° of knee flexion. Senior females presented significantly higher passive shear modulus in RF and VL muscle heads at long muscle length (60° and 90° of knee flexion) than the younger females.

In addition, the RF passive shear modulus could predict 28.3% of the MIVC measured at 60° of knee flexion across the age groups. A bout of eccentric exercise induced significant increase of passive shear modulus of RF muscle at long length (90° of knee flexion). Slight but significant decrease in VL passive shear modulus was observed at 30° and 60° knee flexion. No change was observed in VM muscle. The decrease in MIVC at 48 H was negatively correlated with the RF passive shear modulus measured at 90° of knee flexion before the exercise. The RF passive shear modulus was significantly higher for 35% and 42% in young males when it was stretched to a long length (60º and 90º knee flexion, respectively) than young females.

The active shear modulus of VM, RF, and VL at 30º knee flexion in senior females was higher than young females by 10%, 20%, and 21%, respectively. Young females exhibited greater active shear modulus of the three muscles than young males at 60º and 90º knee flexion ($p = 0.024$ and $p < 0.001$, respectively).

From the results of the four studies, the following conclusions could be drawn. (1) In response to passive lengthening beyond the slack angle, the RF muscle head of the quadriceps femoris muscle shows dissimilar response than the VL and VM muscle heads. (2) Dissimilar modulation on muscle stiffness associated with gender and in response to aging and a bout of eccentric exercise is observed when the muscle heads are positioned beyond its slack angle (at a stretched length). More specifically, the VL and RF muscles but not the VM muscle present greater passive stiffness in senior females than young females; and the RF muscle head shows greater passive stiffness in the young males than young females. A bout of eccentric exercise induces significant increase in the RF passive stiffness. (3) In addition, passive muscle stiffness is associated with muscle function. First, the passive muscle stiffness of RF is one of the predictors for isometric knee extension force; Second, stiffer RF may induce greater muscle damage associated with a bout of eccentric exercise at 48 H after exercise. Taken together, “appropriate” passive stiffness of the RF muscle stiffness is essential for the function of the quadriceps femoris on isometric and eccentric muscle work. Active muscle stiffness associated with submaximal isometric contraction is similar among the three superficial muscle heads of the quadriceps femoris muscle.

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TABLE OF CONTENTS

CERTIFICATE OF ORIGINALITY i

ABSTRACT ii

PUBLICATIONS ARISING FROM THE THESIS v

ACKNOWLEDGEMENTS vi

TABLE OF CONTENTS vii

LIST OF ABBREVIATIONS viii

LIST OF FIGURES xiv

LIST OF TABLES xvi

Chapter 1 Literature review 1

1.1 Introduction 2

1.1.1 The quadriceps femoris muscle 2

1.1.2 Dissimilarity on individual heads of quadriceps femoris muscle 2

1.2 Muscle stiffness and muscle length 4

1.2.1 Passive muscle stiffness 4

1.2.2 Active muscle stiffness 6

1.3 Extrinsic factors on muscle stiffness 8

1.3.1 Gender effect on passive muscle stiffness 8

1.3.2 Gender effect on active muscle stiffness 9

1.3.3 Age-related change in passive muscle stiffness 9

1.3.4 Age-related change in active muscle stiffness 12

1.3.5 Exercise-induced modulation on muscle stiffness 12

1.4 Measurements of muscle stiffness 19

1.5 Rationales of this study 23

1.6 Objectives of the study 24

1.7 Hypotheses of the study 25

1.8 The design of the study 26

Chapter 2 Stiffness of individual quadriceps muscle assessed using ultrasound shear wave elastography during passive stretching 28

2.1 Abstract 29

2.2 Introduction 30

2.3 Methods 32

2.3.1 Participants 32

2.3.2 Passive stretching 32

2.3.3 Muscle shear modulus measurements 32

2.3.4 Experimental protocol 33

2.3.5 Data analysis 34

2.3.6 Statistical analysis........34

2.4 Results........35

2.4 Discussion........39

2.5 Conclusion........41

Chapter 3 Age-related alteration in passive and active muscle stiffness in healthy young and senior females........43

3.1 Abstract........44

3.2 Introduction........46

3.3 Methods........49

3.3.1 Participants........49

3.3.2 Passive muscle stretching........50

3.3.3 Muscle stiffness measurement........50

3.3.4 Six-minute walk test (6MWT)........51

3.3.5 Five times sit-to-stand test (FTSST)........52

3.3.6 Maximal isometric voluntary contraction (MIVC) test........52

3.3.7 Test protocol........53

3.3.8 Data analysis........54

3.4 Results........55

3.4.1 The reliability of the passive and active shear modulus........55

3.4.2 Age-related modulation on passive muscle shear modulus 55

3.4.3 Age-related modulation on active muscle shear modulus 59

3.4.4 The muscle performance 63

3.4.5 Relationship between passive muscle stiffness, muscle strength, and function 63

3.5 Discussion 68

3.6 Conclusion 74

Chapter 4 Gender effect on passive and active stiffness of individual quadriceps femoris muscle 75

4.1 Abstract 76

4.2 Introduction 77

4.3 Methods 78

4.3.1 Participants 78

4.3.2 Passive muscle stretching 78

4.3.3 Muscle stiffness measurement 79

4.3.4 Maximal isometric voluntary contraction (MIVC) test 80

4.3.5 Test protocol 80

4.3.6 Data analysis 81

4.4 Results 82

4.5 Discussion 86

4.6 Conclusion 88

Chapter 5 Relationship between pre-exercise muscle stiffness and muscle damage induced by eccentric exercise 90

5.1 Abstract 91

5.2 Introduction 93

5.3 Methods 95

5.3.1 Participants 95

5.3.2 Study design 96

5.3.3 Maximal isometric knee extension force assessment 97

5.3.4 Muscle stiffness measurement 98

5.3.5 Muscle soreness assessment 99

5.3.6 Interventions 99

5.3.7 Statistical analysis 100

5.4 Results 100

5.5 Discussion 106

5.6 Conclusion 110

Chapter 6 Summary and conclusions 111

6.1 Rationale of the study 112

6.2 Summary of the studies 112

6.3 Clinical Implication of the research findings 115

6.4 Limitations of the Study 116

6.5 Significance of the Study 117

REFERENCE 119

APPENDIX 142

LIST OF ABBREVIATIONS

Abbreviations	Full names
6MWT	Six-minute walk test
CK	Creatine kinase
DOMS	Delayed onset muscle soreness
ECM	Extracellular matrix
EMG	Electromyography
FTSST	Five times sit-to-stand test
MIVC	Maximal isometric voluntary contraction
MRE	Magnetic resonance elastography
MRI	Magnetic resonance imaging
ICC	Intraclass correlation coefficient
RF	Rectus femoris
ROI	Region of interest
SSI	Supersonic shear wave imaging
US	Ultrasound
VI	Vastus intermedius
VL	Vastus lateralis
VM	Vastus medialis
VMO	Vastus medialis obliquus

LIST OF FIGURES

Figure 1. 1 The length-tension curves of gastrocnemius muscle measured on the same day of three separate weeks 6

Figure 1. 2 Postulated steps resulting in muscle damage after eccentric exercise........ 13

Figure 1. 3 Active length-tension curves of 3 parts of medial gastrocnemius muscle of the anesthetized cat 16

Figure 2. 1 Typical example of maps of shear modulus obtained during passive knee flexion 36

Figure 2. 2 Averaged passive shear modulus-angle curve obtained during passive knee flexion. 37

Figure 2. 3 Typical examples of visual determination of slack angle from the shear modulus-angle curve of VM, RF, and VL 38

Figure 3. 1 The measurement of the muscle stiffness on the rectus femoris muscle at 90º of knee flexion........ 51

Figure 3. 2 The passive shear moduli of VM, RF, and VL muscles of the young and senior groups at different knee angles 58

Figure 3. 3 The comparison of active shear modulus of VM, RF, and VL muscles between groups........ 61

Figure 3. 4 The angle effect on active shear moduli among VM, RF, and VL muscles........ 62

Figure 3. 5 The relationship between passive shear modulus of rectus femoris muscle and the peak torque of maximal isometric voluntary contraction 67

Figure 4. 1 The comparison of passive shear modulus of VM, RF, and VL muscles between males and females 84

Figure 4. 2 The comparison of active shear modulus of VM, RF, and VL muscles between males and females 85

Figure 5. 1 The study design program. 97

Figure 5. 2 Muscle shear moduli measured at pre-, post-, and at 48 H post-intervention 104

Figure 5. 3 Typical elastography images of RF shear modulus measured at 90° of knee flexion 105

Figure 5. 4 Plotting of peak torque index and passive shear modulus of RF at 90° of knee flexion in participants receiving eccentric exercise 106

LIST OF TABLES

Table 1. 1 The comparisons of passive muscle stiffness between young and aged muscles... 11

Table 3. 1 The demographic information of participates (means ± SD). 49

Table 3. 2 The ICC values of within-day and between-day reliability for passive and active shear modulus. 56

Table 3. 3 Comparison between the young and senior adults on passive shear modulus of the superficial heads of the quadriceps muscle.. 57

Table 3. 4 The active shear modulus of the VM, RF, and VL muscles when the loading was 30% MIVC at three knee angles. 60

Table 3. 5 The comparison of MIVC, 6MWT, and FTSST between young and senior groups. 63

Table 3. 6 Correlation coefficients among passive muscle shear modulus of the superficial heads of the quadriceps, MIVC, and muscle strength. 65

Table 3. 7 Correlation coefficients (p) among passive muscle shear modulus of the superficial heads of the quadriceps and functional performance... 66

Table 4. 1 The MIVC of keen extensors between males and females at three tested knee angles 83

Table 5. 1 The demographic information of participants in two groups 96

Table 5. 2 Peak isometric knee extensor torque and muscle soreness before (pre), after (post), and 48 hours (48 H) after exercise. 102

Chapter 1

Literature review

1.1 Introduction

The quadriceps femoris muscle relies on the synchronized action of its four muscle heads and controls the position and movement of the patella–femoral and tibio–femoral knee joints. Dissimilarity on structure and function have been reported on individual heads of the quadriceps femoris muscle. Divergence on their mechanical properties might cause mal-alignments of the patella–femoral and tibio–femoral knee joints and might affect muscle function and performance.

1.1.1 The quadriceps femoris muscle

The quadriceps femoris muscle is a large muscle group that covers the front and sides of the thigh. It contains three superficial heads, vastus medialis (VM), rectus femoris (RF), and vastus lateralis (VL), which are located in the medial, middle, and lateral thigh respectively and one deep head called vastus intermedius (VI). The four heads converge into an extremely powerful tendon on the distal part of thigh and then cover patella and ultimately insert into the tuberosity of the tibia (Susan 2015). The primary function of the muscle group is to extend the knee joint; the RF muscle also contributes to flex the hip joint. Additionally, these muscles play an essential role in the stabilization of the knee joint and are critical to protect the joint structures from injury (Fink et al. 2007).

1.1.2 Dissimilarity on individual heads of quadriceps femoris muscle

The four muscle heads function together but each muscle head presents dissimilarity on structure, function, and mechanical properties.

The medial component of the superficial heads, VM, is separated into proximal and distal parts (Lieb and Perry 1968). The distal portion is called vastus medialis obliquus (VMO), with a mean angular orientation of 52.2° to the femur axis. The VMO muscle is considered to be an important muscle because of its role in stabilizing the medial patella during knee extension (Croisier et al. 2007; Waligora et al. 2009), and a weak VMO leads to the lateral shift of patellar while the knee extends to final 15° range of motion (Sakai et al. 2000). The muscle with smallest cross-sectional area (Arnold et al. 2010), RF, is a biarticular muscle across the hip and knee joint with the functions of knee extension and hip flexion. The lateral part of thigh is mainly composed by the VL, which has the largest size and generates the greatest force among the four heads (Ichinose et al. 1997). The VI, sitting underneath the RF, is too deep to record its activity separately and clearly investigate its additional functions (Watanabe and Akima 2009).

The proportion of muscle fibers is different among individual muscle heads. The VL and RF muscle heads have higher proportion of type II (55–70%) than type I muscle fibres (Garrett et al. 1984; Johnson et al. 1973; Staron et al. 2000). The VM, however, contains similar proportion of type I and type II muscle fibers (Garrett et al. 1984). As has been well recognized muscle gets atrophy with aging, which is accompanied by force fall. Specimen and whole muscle investigation showed that aging-induced atrophy of muscle did not result from the decrease in number of type I and type II fiber (Deschenes 2004; Lexell et al. 1988; Morse 2011; Wust et al. 2008) , but the size loss of muscle fibers, especially type II muscle fibers (Andersen 2003; Lexell et al. 1988). The type II muscle fibers appeared to have a 26–29% loss in cross sectional area from young to aged individuals, but such change could not be observed in the type I muscle fibers (Lexell et al. 1988; Nilwik et al. 2013). Similarly, Andersen (2003) reported that in very old subjects, the size of the type II and I muscle fibers were reduced by 75% and 43%, respectively, compared with young subjects. Interestingly, the force of aged quadriceps femoris muscle was 40% less than young while the thickness of muscle only decreased 23% (Ditroilo et al. 2012) and the muscle fiber size is 20% smaller than that of young quadriceps (Martin-San Agustin et al. 2018). Based on this evidence, it seems aging-induced loss of force cannot be explained by just one reason.

In addition, the injury rate among the four muscle heads is extremely unequal (Cross et al. 2004). Cross et al. (2004) investigated 25 clinical quadriceps strains using magnetic resonance imaging (MRI) and confirmed that the majority (15) of strains occurred within RF muscle; the tendon of RF and RF peripheral area; some happened to VI muscle; one in VL muscle; and none in VM muscle. It has been widely considered that the high susceptibility to muscle injury of individual muscle is due to greater passive and active muscle stiffness (Krivickas and Feinberg 1996; McHugh et al. 1999; Wilson et al. 1991); thus it is essential to better understand muscle stiffness of the muscle heads of the quadriceps muscle.

1.2 Muscle stiffness and muscle length

The muscle stiffness is one of the most important elements of mechanical properties for skeletal muscles. It is physiologically critical for a muscle to execute its contractile function, maintain posture, and produce joint motion with normal kinematic of its associated joints (Granata et al. 2002b; Wang et al. 2017). The muscle resistance responding to being passively stretched from short to long length is called passive muscle stiffness (Gajdosik 1995). When a muscle performs active contractions, it generates active stiffness (Gennisson et al. 2005). Excessively high resting muscle tension (passive muscle tension) and imbalance in active muscle stiffness might induce some common muscle and joint problems (Magnusson et al. 2000; Witvrouw et al. 2003).

1.2.1 Passive muscle stiffness

Muscle maintains its length against external forces by increasing passive stiffness. The passive stiffness is referred to as passive tension, or passive elastic force, passive elasticity, passive muscular compliance, passive extensibility, resting tension, or passive muscle tone (Hug et al. 2013; Schleip et al. 2006). Passive stiffness is adopted to depict the mechanical property of skeletal muscle in this study.

When a muscle is progressively lengthened from its shortened state, the passive muscle stiffness gradually increases both in animal (Davis et al. 2003; Nightingale et al. 2003; Pousson et al. 1991; Winters et al. 2011) and human (Gajdosik et al. 2004; Herbert et al. 2011; Hug et al. 2013; Koo et al. 2014). This change is termed a tension-length relationship (Kubo et al. 2001; Schleip et al. 2006). Figure 1.1 shows a typical piecewise tension-length curve of a skeletal muscle. During passive lengthening, muscle stiffness remains unchanged initially but shows exponential increase after a critical point. The length of muscle fascicle at which the muscle begins to increase passive stiffness is called slack length (Hug et al. 2013; Lacourpaille et al. 2013). The joint angle at which passive muscle stiffness starts to develop is termed a slack angle (Hoang et al. 2005; Koo et al. 2014). Muraoka et al. (2004) considered that the force initiated from a muscle could be delivered to the bones for stabilization or initiation of joint movement only when the muscle is elongated beyond its slack length. Although the muscle heads within a muscle group work as a group, the slack length of each muscle heads is not always the same. Hirata et al. (2002) demonstrated that the heads of the triceps surae exhibit different slack angles during passive dorsiflexion with medial gastrocnemius muscle at 20º plantar flexion, lateral gastrocnemius muscle at 15º plantar flexion, and soleus at 2º dorsiflexion.

Similar to the triceps surae, the quadriceps femoris muscle is composed of bi-articular (RF) and monoarticular (VM, VL, VI) muscle heads. The four muscle heads are of different structure and function. Whether disparity on muscle slack length exists among the heads of the quadriceps femoris muscle is unexplored. Such information will develop a better understanding of muscle physiology and is clinically important. This is because any difference in the slack length of individual muscle heads within the quadriceps femoris muscle might induce an imbalance of force transition on its associated joints, such as the patellofemoral and tibio–femoral joints.

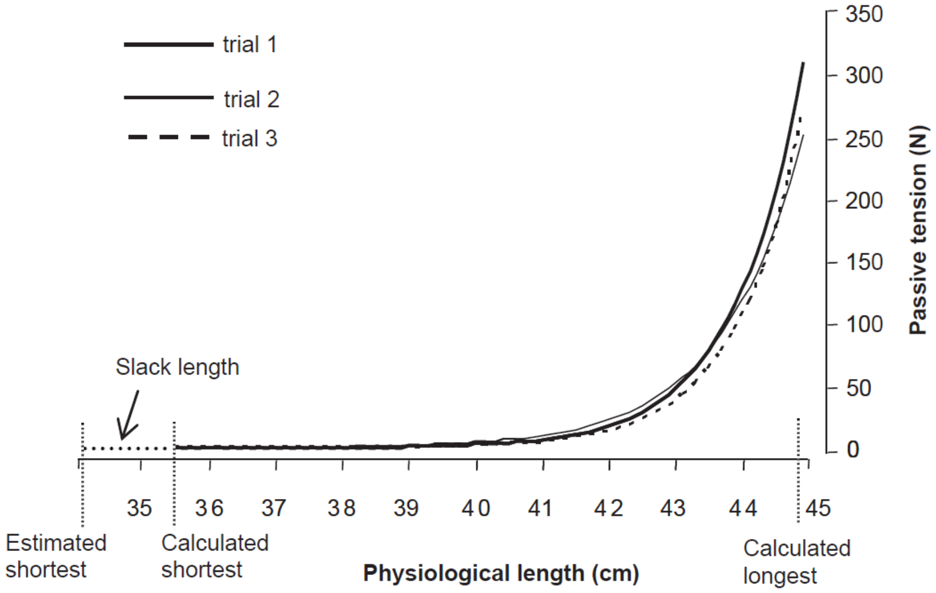

Figure 1. 1 The length-tension curves of gastrocnemius muscle measured on the same day of three separate weeks (Hoang et al. 2005).

1.2.2 Active muscle stiffness

Active muscle stiffness is a normal physiological response to the production of force during muscle contraction. A linear regression was found between active stiffness and electromyogram (EMG) activity (Kim et al. 2018; Nordez and Hug 2010). Also, active stiffness of muscle increased linearly with the gradual increase of isometric force output for the upper limb muscles such as the biceps brachii muscle, abduction digital muscle, and first dorsal interosseous muscle; it also included the lower limb muscles such as the vastus medialis muscle, rectus femoris muscle, vastus lateralis muscle, tibial anterior muscle, and hamstring muscles

(Alfuraih et al. 2019; Ates et al. 2015; Bouillard et al. 2011; Kim et al. 2018; Nordez and Hug 2010; Sasaki et al. 2014; Yoshitake et al. 2014). Consistent findings from these studies indicate a relationship between muscle active stiffness and muscle force output.

However, muscle active stiffness reached a different level when the muscle performed diverse functions at several joint angles (Shinohara et al. 2010). More specifically, when healthy medial gastrocnemius muscle performed 30% of maximal voluntary contraction (MIVC) with the knee in flexion, muscle stiffness increased from 16 kPa to 41 kPa (of 156%). When the knee was in full extension and the muscle was in a more stretched position, a greater increase (to 225 kPa) in muscle stiffness was observed (Shinohara et al. 2010). The findings suggest that the active stiffness increases when muscle contracts at the same intensity at increased muscle length. The increase in muscle stiffness is possibly due to overlapping of sarcomeres that could be affected by the starting length of the muscle (Ford et al. 1981).

Taken together, passive and active muscle stiffness are related to muscle length. An increase in passive muscle stiffness starts when the muscle continues to lengthen beyond its slack length. Whether the muscle heads of the quadriceps femoris muscle would have dissimilar slack length is unknown. A higher active muscle stiffness is required in reaching similar level of force output when a muscle is positioned in its lengthened than shortened position. Alteration on muscle length association with joint position might be dissimilar on the muscle heads with different pennation angles (Arnold et al. 2010). Disproportional changes on the active stiffness of the superficial muscle heads of the quadriceps femoris might induce muscle imbalance when the muscle is positioned in its lengthened position. This is another important issue to explore.

1.3 Extrinsic factors on muscle stiffness

Besides the muscle length could influence the passive and active muscle stiffness, other subjects' characteristics including age, gender, exercise, different muscle could affect the muscle stiffness as well. The following context presents current information on how gender, age, and exercise and muscle stiffness of the quadriceps femoris muscle are related.

1.3.1 Gender effect on passive muscle stiffness

Gender has been believed to be a factor that affects the passive muscle stiffness. Measured by passive torque, the passive stiffness of medial gastrocnemius muscle was 44% greater in males than females when the muscle was passively stretched beyond 25º of dorsiflexion (Morse 2011). Similarly, assessed by ultrasound shear wave elastography, the muscle stiffness of gastrocnemius and masseter muscles in males was higher than that of females (Arda et al. 2011; Yoshida et al. 2016). On the contrary, for biceps brachii muscle, it was revealed by ultrasound shear wave elastography that there was a trend that females showed higher muscle stiffness than males (Eby et al. 2015).

However, another study carried out by Brandenburg et al. (2015) showed that there is no difference of passive muscle stiffness on lateral gastrocnemius muscle between normally developed boys and girls of 2–12 years. Even for the hamstring muscle, which is widely believed that males displayed significantly greater active and passive stiffness than females (Blackburn et al. 2004), the shear modulus calculated from the ratio of stress to strain did not show any difference across genders (Blackburn et al. 2009). In regard to the quadriceps femoris muscle, when the isolated VM, RF, and VL muscle bellies were measured by tensiomyography, only RF muscle was observed to have a higher stiffness in males than females (Martin-San et al. 2018). Therefore, the effect of gender on passive muscle stiffness is inconclusive. More essentially, whether the gender effect is muscle-head specific is unexplored. This is important because tightness of rectus femoris muscle was considered as one of the causes for anterior knee pain (Waryasz and McDermott 2008).

1.3.2 Gender effect on active muscle stiffness

Unlike passive stiffness, gender difference is consistently reported on active muscle stiffness. When the participants contracted at loadings of 6 kg and 20% maximal force, results revealed that active muscle stiffness of females was 27–43% less than that of males (Granata et al. 2002b). The same research group also demonstrated greater active leg stiffness in males than females while they performed functional hopping activity (Granata et al. 2002a). Another study also reported higher active stiffness of hamstring in males compared with females when contracting at loading of 10% total body mass (Blackburn et al. 2004). The active muscle stiffness was calculated from complex formulas and represented active mechanical properties of not only muscle, but multiple tissues including muscles, nerves, ligaments, and vessels. Based on shear wave elastography, Botanlioglu et al. (2013) also demonstrated the active muscle stiffness of males was higher than that of females, which was accompanied by larger force produce while the participants performed maximal isometric knee extension. However, due to a linear relationship between shear modulus and force of muscle (Ates et al. 2015; Bouillard et al. 2012; Sasaki et al. 2014; Yoshitake et al. 2014), higher active stiffness might depend on greater force output of males. Therefore, further study is required to assess gender difference on active muscle stiffness with the contracting intensity.

1.3.3 Age-related change in passive muscle stiffness

Many researchers have explored age-related change of passive muscle stiffness in animals and humans. In rats, increase in passive muscle stiffness was observed in aged muscles (Alnaqeeb et al. 1984; Rosant et al. 2007). In human studies (Table 1.1), the rectus femoris and gastrocnemius muscles in the lower limb (Akagi et al. 2015; Ditroilo et al. 2012) and the bicep brachii muscle in the upper limb (Eby et al. 2015) were reported higher stiffness in aged participants when the muscles were evaluated in a lengthened/stretched position. On the other hand, when the muscles were assessed in a relaxed/shortened position, there was no significant relationship between passive muscle stiffness and age (Arda et al. 2011; Ditroilo et al. 2012; Domire et al. 2009; Hinman et al. 2002). Such observation suggests that age-related increase in passive muscle stiffness might only be detected when muscle is in its lengthened/stretched position. However, Akagi et al. (2015) and Alfuraih et al. (2019) reported opposite results that a lower passive muscle elastic modulus in the aged quadriceps and lateral gastrocnemius muscle compared to young adults. The age-related decrease in gastrocnemius but not soleus muscle which are the muscle heads of the calf muscle was reported by Akagi et al. (2015). That means the influence of aging on passive muscle stiffness was muscle-head different. Similarly, the quadriceps femoris muscle is composed of four muscle heads. RF muscle is a biarticular muscle; VL muscle is the biggest and most powerful of the four muscle heads; and VM muscle is mainly for joint stabilization. Whether the effect of age on passive stiffness is increase or decrease in these muscle heads is different remains unexplored.

Table 1. 1 The comparison of passive muscle stiffness between young and aged muscles.

Author (year)	Muscle	Outcomes		Muscle length during measurement
Ditroilo et al. (2012)	RF	Old > young	-	Supine, 70° knee flexion
Eby et al. (2015)	BB	Old > young with elbow in extension	No difference with elbow in flexion	Elbow flexion: 90° Elbow extension: 0°
Arda et al. (2011)	MG Masseter muscles Supraspinatus	-	No difference	Prone with feet hanging over the edge of the examination bed relaxed
Domire et al. (2009)	TA	-	No difference	20° ankle plantar flexion
Wang et al. (2014)	VI	-	No difference	Sit, 60° knee flexion
Akagi et al. (2015)	RF, LG, SOL	Young > old in RF and LG	No difference in SOL	0° hip and knee flexion for RF 20° ankle plantar flexion for LG and SOL
Yoshida et al. (2016)	MG	-	No difference	15° knee flexion and 15° ankle plantar flexion

BB: Biceps brachii, VL: vastus lateralis, RF: rectus femoris, LG: lateral gastrocnemius, MG: medial gastrocnemius, SOL: soleus, TA: tibialis anterior.

1.3.4 Age-related change in active muscle stiffness

It is widely known that the capability of force generation of muscle decreased with aging. For example, the knee extensor strength of aged males and females was 32% and 48% lesser than young males and females respectively (Samuel et al. 2012). A few studies have investigated the influence of age on active stiffness of a contracting muscle. When extending the knee joint at 20% of maximal isometric voluntary contraction (MIVC), young adults showed higher active stiffness in the vastus medialis muscle than older adults (Debernard et al. 2011). When the contracting intensity increased to 100% MIVC, the deep heads of quadriceps femoris muscle, vastus intermittent muscle of young similarly presented greater active stiffness compared to the elderly subjects (Wang et al. 2017). However, when performing ankle planter flexion at a similar level of absolute torque rather than the percentage of MIVC, young and elderly adults displayed similar active stiffness (Blanpied and Smidt 1993). This inconsistent result seems to be related with the disparity standard of force during comparison. When using percentage of MIVC, the absolute torque in young adults is greater than elderly adults due to progressive muscle weakness with age (Jubrias et al. 1997; Samuel et al. 2012). This greater absolute force production could possibly be the reason for higher active stiffness in young adults when contracting at the same percentage MIVC. But whether there exists any difference of active stiffness between young and elder adults awaits further investigation.

1.3.5 Exercise-induced modulation on muscle stiffness

While the force generated in muscle is less than an external resistance imposed on muscle, the contracting muscle is lengthened gradually, causing an eccentric contraction (LaStayo et al. 2003). It has been well established that an eccentrically contracted muscle is susceptible to damage of its contractile and cytoskeletal constituents (Cleak and Eston 1992; Friden and Lieber 1998; Raastad et al. 2010). The direct measurement of the damage through histology is technically impossible. The magnitude of muscle damage is usually assessed by indirect markers, such as reducing in force production, delayed onset muscle soreness (DOMS), and increase in passive muscle stiffness.

1.3.5.1 Mechanism of eccentric contraction induced muscle damage

A series of events was postulated to be involved in the muscle damage process (Figure 1.2) (Proske and Allen 2005). During high force repeated eccentric contractions, overstretched sarcomeres start to become excessively lengthened from frailest to strongest sarcomeres. Part of the excessively lengthened myofilaments of the sarcomeres might lose the ability to interdigitate again, which leads to disruption of the sarcomeres. Once the number of disrupted sarcomeres reaches to a point at which membranes in muscle including the sarcoplasmic reticulum, transverse tubules, or the sarcolemma gets injured. This injury is followed by an uncontrolled release of Ca^{2+} into sarcoplasm, activating the subsequent steps of muscle damage.

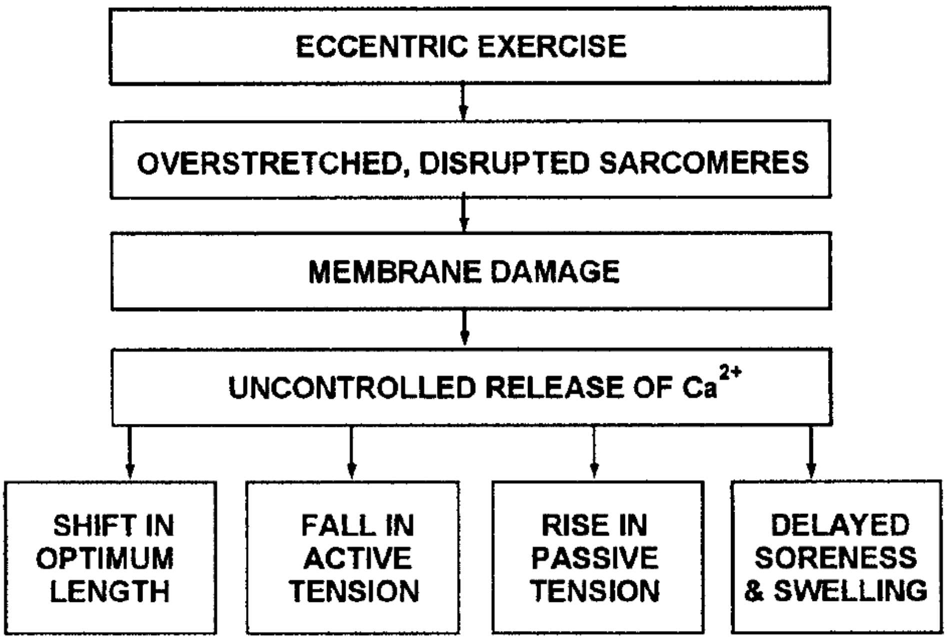

Figure 1. 2 Postulated steps resulting in muscle damage after eccentric exercise (Proske and Allen 2005).

1.3.5.2 Eccentric exercise-induced modulation in passive stiffness

It has been widely documented that muscles became 21–60% stiffer immediately after a bout of eccentric exercise compared with baseline (Chleboun et al. 1998; Green et al. 2012; Hoang et al. 2007; Howell et al. 1993; Lacourpaille et al. 2014; Pournot et al. 2016; Whitehead et al. 2001). The increased passive muscle stiffness reached its maximum at 1–4 days post-exercise and gradually subsided in 1–4 weeks (Jones et al. 1987). Most of these studies measured passive stiffness by torque-angle relationship that represents not only the passive stiffness of muscle but also extensibility of the nerve, vessels, and tendon tissues. Recently, magnetic resonance elastography (MRE) or ultrasound elastography were developed to provide direct measurements on passive muscle stiffness.

Based on real-time tissue elastography, Yanagisawa et al. (2011) reported that after eccentric contractions of elbow flexors, the passive stiffness of biceps branchii muscle increased immediately and recovered to pre-exercise level within 30 minutes; for gastrocnemius muscle, the passive stiffness appeared to have around a 20% increase after eccentric exercise and peaked at 48 hours post-exercise when measured using MRE (Green et al. 2012). Using ultrasound elastography, there was a similar increase (by 28%) that was observed on the gastrocnemius muscle, but the change could not last 48 hours post-exercise (Guilhem et al. 2016). Most importantly, exercise-induced change in muscle stiffness may be different between individual muscles that belong to the same muscle group such as the calf muscles (Green et al. 2012). Green et al. (2012) used MRE to assess the changes of passive muscle stiffness of the gastrocnemius and soleus muscles after 1 session of a calf-raising exercise. The gastrocnemius muscle was found to have significant increase in muscle stiffness but not the soleus muscle. However, a totally opposite result has been recently reported by Andonian et al. (2016). The authors measured the passive stiffness of superficial heads of quadriceps femoris muscle before, halfway through, immediately post, and 48 hours post an extreme mountain ultra-marathon mainly composed of low-intensity eccentric exercise. They found the passive stiffness of all measured muscle heads experienced a significant decrease immediately post the trace, which was followed by a partial recovery at 48 hours after the race. The authors considered the contradictory result was related to the relatively low eccentric intensity and long duration during mountain ultra-marathon because eccentric exercise-induced muscle damage is high intensity and duration dependent (Barroso et al. 2011; Jamart et al. 2012; Nosaka and Newton 2002). Such information indicates that change in passive muscle stiffness is muscle dependent and muscle-head specific within the same muscle group.

These findings show that the increase in passive muscle stiffness might be muscle dependent, muscle head-specific, and time-dependent. Whether similar observation would be detected in the muscle heads of the quadriceps femoris muscle after a bout of eccentric exercise need to be further explored.

1.3.5.3 Eccentric exercise-induced modulation in active stiffness

It has been well established in animals and human beings that the optimal length of muscle at which muscle producing peak active stiffness shifts to a longer direction after a bout of eccentric exercise (Brown and Donnelly 2011; Jones et al. 1997; Lee et al. 2009; Whitehead et al. 2001). From Figure 1.3, it could be seen that eccentric exercise could cause a decrease in active stiffness and the drop was greater when muscle eccentric contracted at a longer length. Leger and Milner (2000) also reported that muscle active stiffness dropped 15% from pre-exercise level on the first day after eccentric exercise at intensity of 50% MIVC and recovered on the second day. Animal study showed similar result with active muscle stiffness dropping around 20% at optimal length and nearly 70% at very long muscle length (Whitehead et al. 2003). The drop in active stiffness is likely to be caused by metabolic fatigue, which may be separated from any muscle damage (Whitehead et al. 2003).

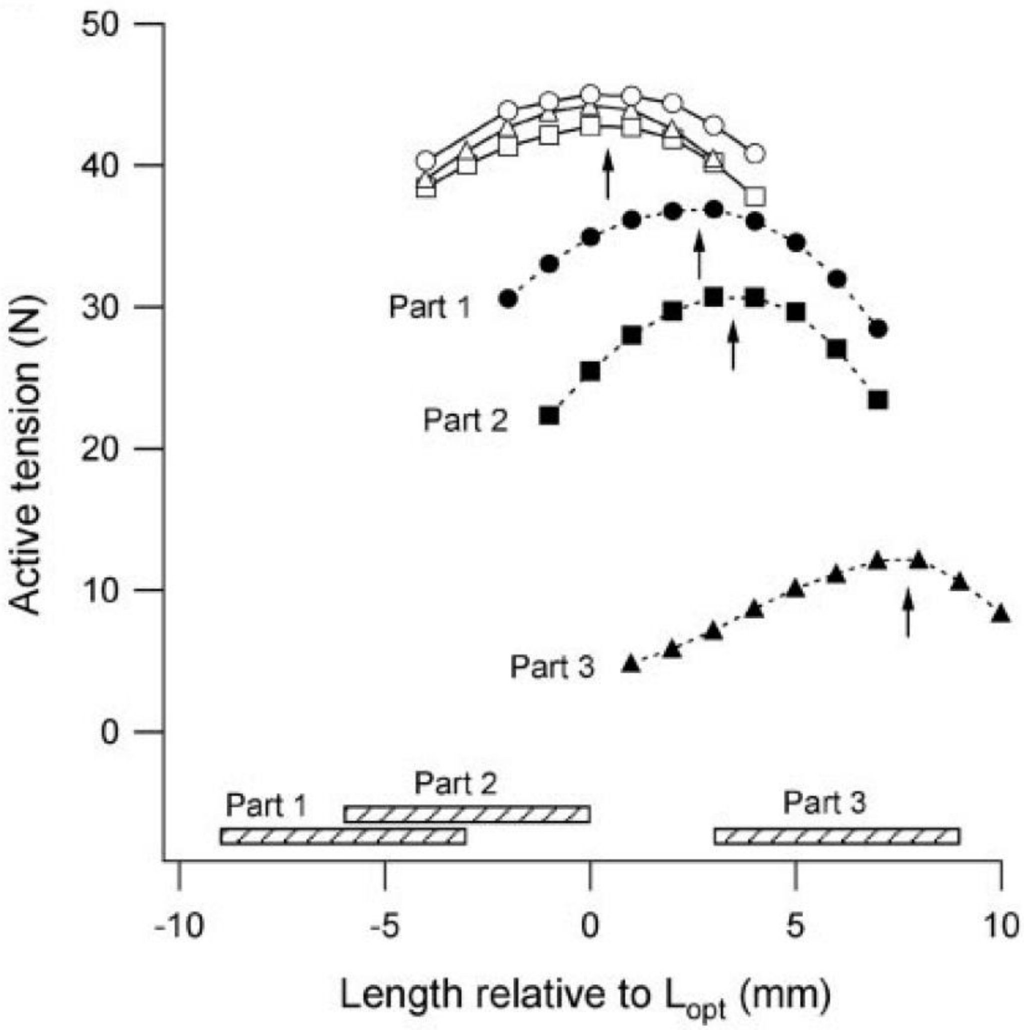

Figure 1. 3 Active length-tension curves of 3 parts of medial gastrocnemius muscle of the anesthetized cat. The optimal length shifted to a longer length after 50 eccentric contractions at three different muscle length ranges. Part 1 (circles) displays the optimal length ranging from -9 mm to -3 mm; part 2 (squares) displays the optimal length ranged from -6 mm to 0 mm; and part 3 (triangles) displays the optimal length ranged from -3 mm to -9 mm (Whitehead et al., 2003).

1.3.5.4 Eccentric exercise-induced reduction in force output

Reducing in force output is another feature of eccentric exercise-induced muscle damage. The reduction in force appeared to have a wide range (from 5% to 30%) (Chen et al. 2011b; Givoni et al. 2007; Hody et al. 2013; Nogueira et al. 2014) and is not homogeneous between muscle groups. Some researchers compared the response of arms and legs to a bout of eccentric exercise and found that elbow flexors are susceptible to greater muscle force reduction than knee extensor (Chen et al. 2011b; Nogueira et al. 2014; Saka et al. 2009) with about 30% versus 5% (Chen et al. 2011b). Therefore, the magnitude of muscle damage in quadriceps femoris muscle was possibly lower than biceps brachii muscle. The large involvement in daily activities like downhill and downstairs of leg muscle may be responsible for the disparity because these activities make the leg muscles expose to eccentric exercise more than arm muscles and produce an adaptation effect against muscle damage induced by eccentric exercise (Jamurtas et al. 2005).

Some parameters have been demonstrated to influence the magnitude of force reduction after a bout of eccentric contractions such as the contraction intensity (Barroso et al. 2011; Chen et al. 2007; Paschalis et al. 2005a), the length of muscle exercise (Newham et al. 1988; Paschalis et al. 2005b), the number of contractions (Chen and Nosaka 2006). However, even when the exercise protocol was the same, the magnitude of force reduction after a bout of eccentric exercise is still highly variable among individuals (Clarkson et al. 2005; Nosaka and Newton 2002). The underlying mechanisms for these individual differences in strength loss are unclear. Muscle extensibility has been proposed as one of the factors that affect the force reduction associated with eccentric exercise (Chen et al. 2011a; McHugh et al. 1999). McHugh et al. (1999) first reported significantly less symptoms of exercise-induced force reduction in participants with more compliant hamstrings than those with stiffer hamstrings. In the same way, Chen et al. (2011a) reported less eccentric exercise-induced force loss after the hamstring muscles performed a flexibility exercise program for eight weeks. In addition, the maximal angle of hip joint flexion that was considered as an index of flexibility of knee flexors was negatively associated with markers of muscle damage such as decrease in muscle force and plasma creatine kinase level (Chen et al. 2011a). However, the range of hip flexion is influenced by many components including the stiffness of the muscle-tendon complex as well as the mobility of the hip joint, which made it difficult to draw a direct relationship between pre-exercise muscle stiffness and the amount of muscle damage. Whether passive muscle stiffness pre-eccentric exercise would affect muscle damage induced by eccentric exercise is still to be explored.

1.3.5.5 Delayed onsets of muscle soreness (DOMS)

The delayed onsets of muscle soreness (DOMS) is characteristic of tenderness or stiffness while the muscle is in palpation and/or movement (Gulick and Kimura 1996). Muscle soreness begins to appear about 1 hour after eccentric exercise, increases intensity, and peaks at 48 hours post eccentric exercise (Hoang et al. 2007; Newham et al. 1983). The average intensity of muscle soreness for quadriceps, however, diverged from less than 1 to 4.5 out of 10 of the visual analogue scale (Chen et al. 2011b; Givoni et al. 2007; Hody et al. 2013; Jamurtas et al. 2005; Nogueira et al. 2014). The soreness is different from other kinds of chronic pain; it could only be perceived when the muscle undergoes mechanical stimulation, including active contraction, passive stretch, or external palpation (Proske and Allen 2005). Researchers have proposed several theories to elucidate the potential mechanics of DOMS. One of the possible causes is an increase in muscle spasm (Cheung et al. 2003; Lewis et al. 2012). Hence, changes in muscle mechanical properties might be one of the causative factors for the DOMS.

1.3.5.6 Increase of CK in plasma

Another muscle damage indicator is a biochemical marker in plasma, creatine kinase (CK). CK raised significantly after eccentric exercise and maintained a peak level 3–5 days post-exercise (Jamurtas et al. 2005; Nosaka and Newton 2002; Nosaka et al. 2002). The magnitude of increase was higher when a muscle was exercised at a lengthened position than a shortened position or with greater repetitions of eccentric contractions (Nosaka and Sakamoto 2001; Nosaka et al. 2002). In addition, the force production during eccentric contractions also has significant effect on the CK level in plasma. This was supported by study of Nosaka and Newton (2002) who compared the effect of 50% and 100% capability of eccentric contractions on plasma CK and found that full effort induced greater increase in CK level.

In summary, the effects of eccentric exercise-induced muscle damage could be affected by exercise intensity (Barroso et al. 2011; Nosaka and Newton 2002), repeated exposure (Allen 2001), length of contractile fibers (Child et al. 1998; Nosaka et al. 2005), upper limb verse lower limb muscles (Chen et al. 2011b; Nosaka and Sakamoto 2001). The muscle mechanical properties, i.e., passive muscle stiffness is one of markers for the eccentric exercise-induced muscle damage. With the recent advancement on imaging technology, direct measurements of muscle stiffness are technically possible. This new technology enables exploration on whether exercise-induced modulation on muscle stiffness is muscle-head specific, and whether change in muscle stiffness is related to other markers associated with exercise-induced muscle damage, such as reduction in force production and DOMS.

1.4 Measurements of muscle stiffness

In order to quantitatively measure stiffness of individual muscle, some imaging techniques have been employed to evaluate this mechanical property of muscle. In 1990, the strain ultrasound elastography was first introduced to measure muscle stiffness based on external tissue compression (Ophir et al. 1991). Several years later, the MRE was applied to measure muscle properties (Muthupillai et al. 1995). The MRE quantitatively evaluates the small displacement of the muscle caused by the propagation of shear wave in muscles provides very good resolution images for both superficial and deep soft tissues (Heers et al. 2003). However, the acquisition time of 20 minutes (Bercoff et al. 2004), the large size, and high expense of MRE limits its wide application. The ultrasound elastography, which is easier to operate and more convenient to be moved around than MRE has been widely applied to measure muscle stiffness without any limitation. The external compression was given by pressuring the skin through the ultrasound probe by hand or a specific oscillator. Together with extra vibrations, including compression through probe or specific oscillator, the ultrasound showed the ability to detect the displacement of muscle by successively capturing images (Gennisson et al. 2013; Klauser et al. 2014). Then the muscle stiffness could be attained through a complicated algorithm (Blackburn et al. 2009; Ditroilo et al. 2012; Wang et al. 2014).

However, the force applied on the skin by handheld probe could not be standardly qualified. In addition, the heavy, huge external vibrators restricted its clinical applicability (Bercoff et al. 2004). Later, acoustic radiation force that induced tissue displacement through focused ultrasound pulse and resulted in a qualitative evaluation of muscle stiffness was introduced to measure tissue stiffness (Nightingale et al. 2001).

Still, there are two disadvantages of this technique. First, it is time consuming to obtain an entire image of the muscle. Second, the local tissue is prone to be heated due to accumulation of energy (Fahey et al. 2006; Palmeri and Nightingale 2004). Then, the shear waves that were produced by the acoustic radiation force became a concern to the researchers (Nightingale et al. 2003). Combined with ultrafast imaging, the propagation speed of shear wave could be calculated and then the muscle stiffness could be estimated. Based on the concept, Bercoff et al. (2004) introduced the supersonic shear wave imaging (SSI) to quantitatively evaluate muscle stiffness in 2004. Briefly speaking, an ultrasound probe creates acoustic waves that pass to different layers of underlying tissues. The acoustic waves then generate mechanical (shear) waves propagating along both sides of tissues. With the same ultrasound probe, the local displacements of tissue induced by the propagation of shear wave are detected by the successive acquisition sequence at a very high-frame rate (up to 20 kHz) (Bercoff et al. 2004). The shear wave propagation velocity could be computed. Based on the fact that the muscle is highly anisotropic tissue (Gennisson et al. 2010), the shear elastic modulus could be calculated through the equation $\mu = \rho v^2$, where ρ is the muscle mass density (1,000 kg/m^3) and v is the shear wave velocity (Bercoff et al. 2004; Gennisson et al. 2005). Then the shear elastic modulus could be used as an index of muscle stiffness.

SSI has been used in measuring changes in muscle stiffness during passive stretching in the lower limb muscles such as the medial gastrocnemius (Hug et al. 2013; Maisetti et al. 2012; Semmler et al. 2002), soleus (Hirata et al. 2015), tibialis anterior (Koo et al. 2014), and biceps brachii muscle in the upper limb (Lacourpaille et al. 2013). In order to ensure the accuracy of ultrasound-based shear wave elastography, the validity and reliability has been determined. When the young person's modulus and the shear modulus of isolated skeletal muscle was measured through slope of the stress–strain curve and shear wave elastography, respectively. The shear modulus measured at mid-belly of a muscle has been validated as the presentation of a whole muscle (r ranged from 0.957 to 0.994) (Eby et al. 2013). Excellent validity (r = 0.996) has also been proved by measuring two tissue-mimicking materials with absolute value of a young person's modulus (Chino et al. 2012). Numerous studies have demonstrated excellent within-session reliability of passive stiffness in a wide range of muscles measured by shear elastography with reliability ranging from 0.871–0.970. (Hatta et al. 2015; Jeon et al.

2018; Lacourpaille et al. 2012; Leong et al. 2013; Nordez and Hug 2010). The between-session reliability of passive muscle was shown to be excellent (with ICC ranging from 0.815–0.950) as well (Lacourpaille et al. 2012; Leong et al. 2013).

The active muscle stiffness induced by muscle contraction could also be quantitatively measured by the SSI. When a greater force is produced by muscle contraction, the muscle becomes stiffer (Bouillard et al. 2011; Granata et al. 2002b; Kim et al. 2018). Based on the evidence, the muscle force could theoretically be indirectly evaluated by measuring active stiffness of a contracting muscle. It has been reported that the shear modulus of a contracting muscle increased with the increment of muscle force in a linear relationship for a wide range of muscles, including the thigh muscles (Ates et al. 2015; Bouillard et al. 2011; Sasaki et al. 2014; Yoshitake et al. 2014). The slope of the line between the force and the shear modulus was negatively associated ($r = 0.83$, $p < 0.01$) with muscle force when biceps brachii muscle contracted at incremental loading up to 60% MIVC (Yoshitake et al. 2014).

More important, Bouillard et al. (2012) measured active muscle stiffness, electromyography (EMG) activity and muscle force during fatigue contractions and found the muscle force estimated through shear modulus was identical with measured muscle force while the accuracy of muscle force estimated from EMG was not as high as shear modulus. As the EMG activity is susceptible to be affected by muscle fatigue (Singh et al. 2007), it is promising to use the shear wave elastography to indirectly assess the muscle force as the linear relationship between active stiffness and force of muscle quantified by SSI that was not influenced by the fatigue (Bouillard et al. 2012). Also, excellent within-session reliability has been reported in the measurement of upper trapezius muscle (Leong et al. 2013), gastrocnemius medial muscle (Jeon et al. 2018), supraspinatus and deltoid muscles (Kim et al. 2018), and biceps brachii muscle (Nordez and Hug 2010). The reliability ranged from 0.93 to 0.98. With a long interval such as 3 days, the repeatability of active muscle stiffness measure by shear wave elastography was reduced to a range of 0.44 to 0.66 (Koppenhaver et al. 2018). Taken together SSI is a valid and reliable tool to quantify passive and active muscle stiffness.

1.5 Rationales of this study

The quadriceps femoris muscle relies on the synchronizing action of its four muscle heads and controls the position and movement of the patella–femoral and tibio–femoral knee joints. Dissimilar on muscle structure, architecture, and function of individual muscle heads of the quadriceps femoris muscle might induce divergence on the modulation on the muscle stiffness.

Difference on the slack angle of the triceps surae has been reported. Divergence on slack angle would occur between the bi-articular (RF) and mono-articular (VL, VM, and VI) muscle heads of the quadriceps femoris muscle. In addition, the medial gastrocnemius muscle shows a higher active muscle stiffness in reaching similar level of force output when the muscle is positioned in its lengthened rather than in its shortened position. As the pennation angle differs on the muscle heads of the quadriceps femoris muscle (Arnold et al. 2010), disproportional changes on the active muscle stiffness on these muscle heads might happen with the knee positioned at different joint angles.

Gender, age, and eccentric exercise are extrinsic factors associated with muscle stiffness. Age-associated increase in passive muscle stiffness has been reported and is found dissimilar on the muscle heads of the triceps surae muscle. The muscle heads of the quadriceps femoris muscle are of different size and fibre types. Aged-related changes on the structures of the muscle heads might induce dissimilar effects on the passive and active stiffness of muscle heads. Gender effect on passive muscle stiffness is inconclusive and its effect on active muscle stiffness needs to be proven further. Increase in muscle stiffness has been reported after a bout of eccentric exercises that is muscle-head specific in the triceps surae muscles (Green et al. 2012). Similar observations would likely be observed in quadriceps femoris muscle. More important, post-exercise reduction in muscle force output is associated with muscle flexibility (Matsuo et al. 2015; McHugh and Nesse 2008). It is possible that muscle stiffness and exercise-induced force reduction are related.

Furthermore, insight to the mechanical properties of single muscle head of the quadriceps femoris muscle could also provide information for health protection associated with aging and post eccentric exercise. If the different modulation on muscle stiffness from age, gender, and eccentric exercise occurs in the muscle heads of the quadriceps femoris muscle, imbalance of function between medial and lateral, anterior and posterior of knee might happen and result in some dysfunction, such as mal-alignment and/or mal-tracking of the patella (Sakai et al. 2000).

1.6 Objectives of the study

The overall objective of the study is to investigate whether modulation on mechanical properties of quadriceps femoris muscle would be muscle-head specific.

Specific objectives of the study are to:

(1) determine the slack angle of individual heads of quadriceps femoris muscle at which the passive stiffness of muscle starts to increase during passive stretching.

(2) assess the effect of age and gender on passive muscle stiffness of individual heads of quadriceps femoris muscle as well as active muscle stiffness when the muscle contracts isometrically.

(3) explore whether a bout of eccentric exercise would change the passive stiffness of individual heads of quadriceps femoris muscle homogenously.

(4) determine whether pre-exercise muscle stiffness is related to the force loss observed after a bout of eccentric knee extensions.

1.7 Hypotheses of the study

The above objectives would be addressed in four studies based on the following research hypotheses:

(1) The slack angles of individual heads of quadriceps femoris muscle would be different. The RF muscle would have smaller slack angle than VL and VM muscles.

(2) a. The passive muscle stiffness of individual heads of quadriceps femoris muscle would be higher in seniors than young adults when the muscles were assessed beyond the slack angle; and the age effect on passive muscle stiffness would be different among the muscle heads.

b. The active muscle stiffness of individual muscle heads of the quadriceps muscle during the same level of isometric contractions would be different in senior and young adults.

c. The passive muscle stiffness is higher in males than females when muscle is assessed beyond the slack angle; the gender effect might differ among the muscle heads.

d. The active muscle stiffness of individual muscle heads of the quadriceps muscle during the same level of isometric contractions might be different across genders.

(3) The passive muscle stiffness of individual heads would increase after a bout of eccentric exercise of quadriceps femoris muscle and the magnitude of change would be different among the muscle heads.

(4) The pre-exercise muscle stiffness might be related to the force loss observed after a bout of eccentric knee extensions.

1.8 The design of the study

The project involved 4 studies.

Study 1: **Stiffness of individual muscle heads of the quadriceps muscle assessed using ultrasound shear wave elastography during passive stretching**

Objective 1: To determine the passive stiffness–angle relationship and the slack angle of VM, RF, and VL muscles.

Objective 2: To explore the difference between VM, RF, and VL muscles on the slack angles.

Study 2: Age-related alteration in passive and active muscle shear modulus in healthy young and senior females

Objective 1: To compare passive shear modulus of individual heads of quadriceps femoris muscle between healthy community-dwelling women.

Objective 2: To compare the active shear modulus of individual heads of quadriceps femoris muscle between healthy community-dwelling women.

Objective 3: To explore the correlation between passive shear modulus of individual heads of quadriceps femoris muscle and muscle performance.

Study 3: Gender-related alteration in passive and active shear modulus of quadriceps muscle in healthy young

Objective 1: To compare passive muscle shear modulus of individual heads of quadriceps femoris muscle between young males and females.

Objective 2: To compare the active muscle shear modulus of individual heads of quadriceps femoris muscle between young males and females.

Study 4: Eccentric exercise-induced modulation on passive and active muscle stiffness of individual muscle heads of the quadriceps muscle

Objective 1: To determine whether a bout of eccentric exercise would change the passive stiffness of individual heads of quadriceps femoris muscle homogenously.

Objective 2: To explore whether pre-exercise muscle stiffness is related to the force generation capacity observed after a bout of eccentric knee extensions.

Chapter 2

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

(Published in Journal of Sport and Health Science)

2.1 Abstract

Purpose: 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 aim of present study was (1) to determine the shear modulus-knee angle relationship and the slack angle of the vastus medialis (VM), Rectus femoris (RF) and vastus lateralis (VL) muscles; (2) to determine whether this differed between the muscles.

Methods: Nine male rowers took part in the study. The shear modulus of VM, 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-angle curve through which the slack angle of each muscle was determined.

Results: The shear modulus of RF was higher than that of VM and VL when the muscles were stretched over 54° (all p values <0 .01). No significant difference was found between the VM and VL (all p values > 0.055). The slack angle was similar among the muscles: 41.3 ± 10.6°, 44.3 ± 9.1° and 44.3 ± 5.6° of knee flexion for VM, 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 works are needed to determine whether changes in muscle stiffness are muscle-specific in pathological conditions or after interventions such as stretching protocols.

Key words: shear modulus; ultrasonography; slack angle; muscle tension; stretch

2.2 Introduction

Flexibility is classically assessed at the joint level by measuring the maximum range of motion or the joint torque during passive motion (Moreau et al. 2016). 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 it exists 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 (Umegaki et al. 2015). Therefore, it is important to assess each individual muscle for a deeper understanding of muscle flexibility and to improve musculo-skeletal 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 (Lacourpaille et al. 2012) that can be considered as a measure of muscle stiffness (Eby et al. 2013). Because it exists a strong linear relationship between muscle stiffness and passive tension when passively stretching muscle (Koo et al. 2013), changes in muscle stiffness measured using SSI can be used to estimate tension changes of muscle responding to passive stretch (Hug et al. 2015). In addition, the SSI method provides an opportunity to estimate the slack angle of individual muscles which is defined as the joint angle beyond which muscle begins to develop passive tension (Hug et al. 2013).

Taking advantage of SSI, changes in muscle stiffness have been estimated during passive stretching in humans, mainly on the medial gastrocnemius (Hirata et al. 2015; Hug et al. 2013, Maisetti et al. 2012), soleus (Hirata et al. 2015), tibialis anterior (Koo et al. 2014) and biceps brachii muscle (Lacourpaille et al. 2013). 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 (Umegaki et al. 2015). Further, Hirata et al. (2015) 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 (Lacourpaille et al. 2013).

It is important to assess the passive behaviour of the quadriceps muscle heads for both clinical and basic science. 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 (Blazevich et al. 2006). As a consequence, along with the hamstrings and triceps surae, quadriceps is one of the three muscle groups that are the most susceptible to be injured in athletes (Chen and Gao 2018). The strain injuries commonly occur in the rectus femoris (RF) muscle (Cross et al. 2004) 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 (Buchanan et al. 2004; Koo et al. 2014), it exists no experimental evidence of this assumption. SSI provides a unique opportunity to test this assumption.

This study was designed (1) to determine the passive stiffness-angle relationship and the slack angle of the vastus medialis (VM), RF and vastus lateralis (VL) muscles; (2) to determine whether this differed 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 making challenging to get reliable measurements.

2.3 Methods

2.3.1 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, Hong Kong Polytechnic University).

2.3.2 Passive stretching

An isokinetic dynamometer was used to impose passive knee flexions (Cybex, Medway, Massachusetts). Before the participant was positioned on the dynamometer, location of the ultrasound transducer was determined on the skin using a waterproof pen for each muscle (VM: 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 ball kicking test was measured. The hip was positioned in neutral position and the presumed axis of the knee rotation was aligned with the axis of the dynamometer.

2.3.3 Muscle shear modulus measurements

An Aixplorer ultrasound scanner (Aixplorer Version 4.2; Supersonic Imagine, Aix-en-Provence, France), coupled with a linear transducer (4-15MHz, Super Linear 15-4; Supersonic

Imagine, Aix-en-Provence, France) 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, this study reported the shear modulus values as the Young's modulus values divided by three (Hug et al. 2015).

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 VM and VL, the optimal transducer location was determined when several muscle fascicles could be seen without disconnection through the image (Blazevich et al. 2006). Because of the complex arrangements of RF fascicles, the transducer was oriented in muscle shortening direction of the lateral component of this muscle (Bouillard et al. 2012; Ema et al. 2013b; Hasselman et al. 1995).

The 2-D maps of muscle shear modulus were captured at 1 sample/s 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 (Kot et al. 2012), the ROI was set as big as possible according to the muscle thickness (about 2.25 cm^2 for VM and VL, 1.5-2.5 cm^2 for RF) to achieve accurate value of passive tension.

2.3.4 Experimental protocol

After a 10 min rest period, 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, i.e. to start the elastography measurements at the start of the loading cycle. Oral instruction was given to the participants to stay relax and avoid any muscle contraction and movement of the leg throughout the passive stretching. The test sequences of the 3 muscles were randomly arranged.

2.3.5 Data analysis

SSI data were exported in mp4 format and sequenced in png. Image processing was performed using a custom Matlab script (MathWorks, Natick, MA). Each image was carefully inspected for artefacts. If artefacts were present in any image, the ROI was reduced in size to remove the artefact from all images within that stretching condition. The coloured 2-D 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 (Hug et al. 2013; Lacourpaille L. et al. 2013). The slack angle determined by this method has been shown to be similar to that calculated from exponential model (Hug et al. 2013; Maisetti et al. 2012) and good intra-session and inter-rater reliability of this visual determination has been reported (Hirata et al. 2015; Hug et al. 2013).

2.3.6 Statistical analysis

Statistical analysis was conducted using 21.0 SPSS software package for Windows (SPSS Inc, Chicago, IL). Data distribution consistently passed the Shapiro-Wilk normality test which showed the slack angles and the shear modulus of the 3 muscles from 9 participants were normal distribution. The variance of passive shear modulus has been conformed to be homogenous of variance. Thus, one-way repeated measures ANOVA (within subject factor: muscle) was used to compare the slack angle between the three 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 $p < 0.05$.

2.4 Results

Representative maps of muscle shear modulus are depicted in Figure 2.1. 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 (Figure 2.2), 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 VM and VL when the knee angle was stretched above 54° (all p values < 0.01). No significant difference was found between the VM and VL in the whole range of motion (all p values > 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 VM (14.5 ± 1.8 kPa; $p = 0.0001$) and VL (12.6 ± 2.6 kPa; $p = 0.00003$).

Figure 2.3 depicts a typical example of visual determination of the slack angle from shear modulus-angle curve of VM, RF and VL. The slack angle was not different between the muscles (main effect of muscle $p = 0.626$) with values of 41.3 ± 10.6° (95% CI: 33.2-49.5°), 44.3 ± 9.1° (95% CI: 37.3-51.3°) and 44.3 ± 5.6° (95% CI: 40.1-48.6°) for VM, RF and VL, respectively.

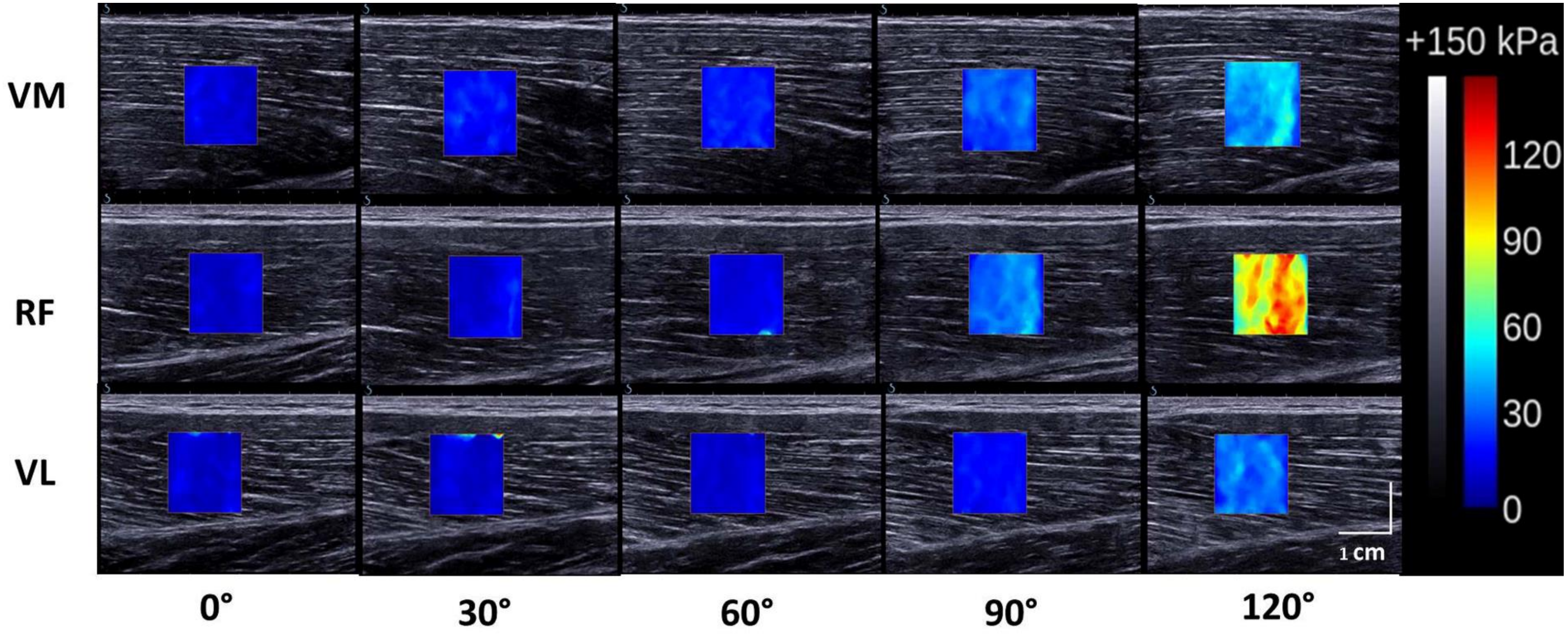

Figure 2. 1 Typical example of maps of shear modulus obtained during passive knee flexion at 0°, 30°, 60°, 90° and 120°. The map of shear modulus was superposed onto a B-mode image, with the color scale depicting graduation of shear modulus. To obtain a representative value, the shear modulus was averaged over the region of interest. While the muscles were stretched, the muscle shear modulus increased.

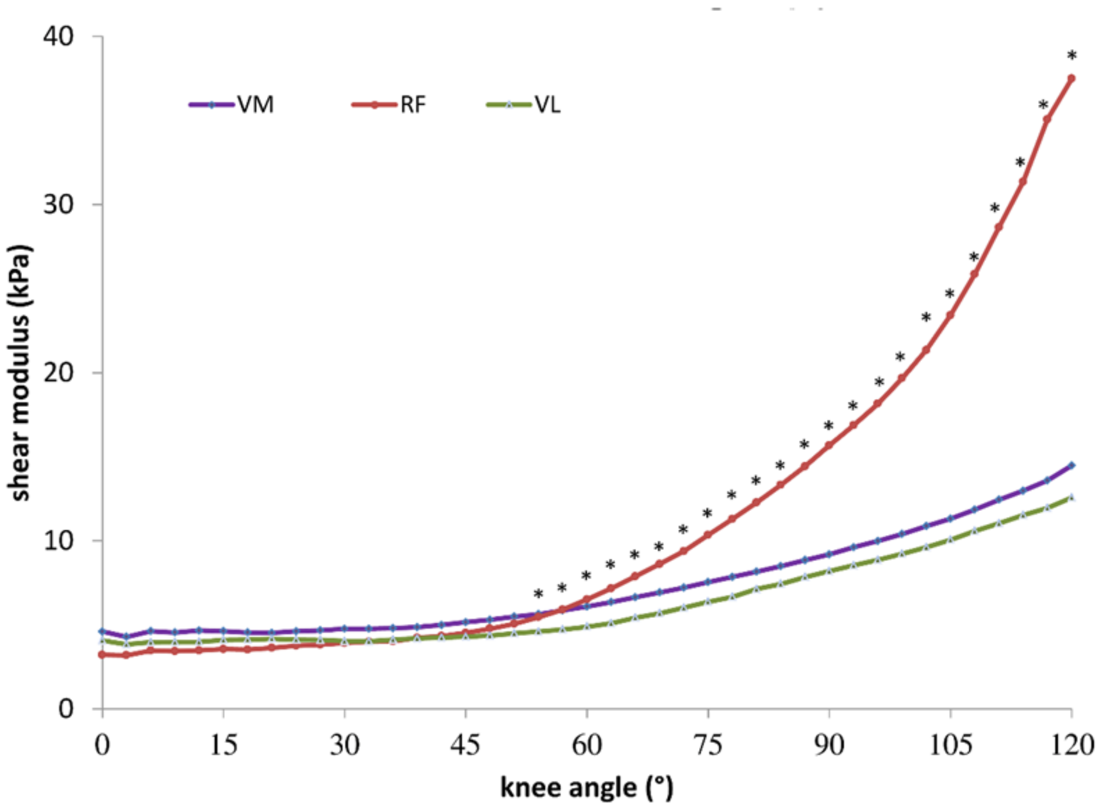

Figure 2. 2 Averaged passive shear modulus-angle curve obtained during passive knee flexion. The standard deviation error bars are not included for clarity. The shear modulus was higher for RF than VM and VL when the knee was passively flexed above 54° (*: $p < 0.01$). No significant difference was found between VM and VL.

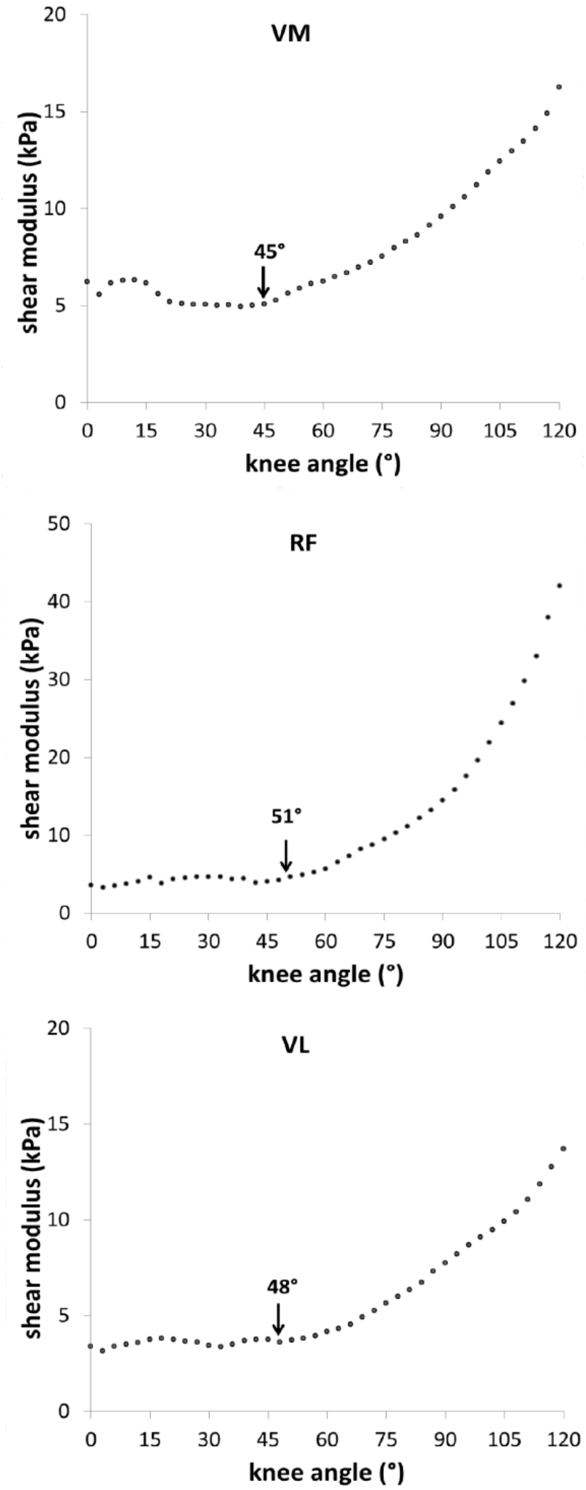

Figure 2. 3 Typical example of visual determination of slack angle from the shear modulus-angle curve of VM, RF and VL. The arrows show the slack angle above which the passive tension begins to increase.

2.4 Discussion

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

To our knowledge, this is the first report to investigate the shear modulus-angle relationship of individual quadriceps muscle during passive knee stretching. As expected, the VM, RF and VL exhibited a similar behaviour, 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 VM and VL over the entire range of knee flexion. This is consistent with finding reported by Bensamoun et al. (2006) who measured the shear modulus of VM and VL at 30° of knee flexion using magnetic resonance elastography (MRE). The present study provides further evidence to expand this earlier observation from one knee angle to almost the whole range of motion (0° to 120° knee flexion). In contrast, shear modulus of RF increased more sharply than VM and VL exhibiting a steeper shear modulus-angle curve above 54° of knee flexion. It is important to consider methodological explanations for this higher RF stiffness. The ultrasound (US) transducer was oriented in muscle shortening direction for RF. However, because of muscle anisoptropy the shear modulus is the highest when the US probe is parallel to the muscle fibres (Gennisson et al. 2010). As such, the fact that the US transducer was not aligned with muscle fascicles for RF was likely to underestimate the shear modulus values rather than overestimate it. Therefore, the conclusion that RF modulus is higher than VL and VM 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 (Gajdosik 2001), 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 (Moreau et al. 2016; Witvrouw et al. 2003). The present results are in accordance with the clinical observation that the much higher prevalence of muscle strain occurred in RF than vastii muscles (Cross et al. 2004).

Although RF exhibited a higher passive stiffness than VL and VM above 54° of knee flexion, the slack angle was similar across the three investigated muscles (about 40° of knee flexion). The similarity in slack angle among the three superficial heads of quadriceps muscle indicates 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 (Lacourpaille et al. 2013). In contrast, Hirata et al. (2015) 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 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 (Buchanan et al. 2004; Koo et al. 2014). As the optimal angle for the knee extensor muscle has been reported to be 70°-80° of knee flexion (Ichinose et al. 1997; Marginson and Eston 2001; Pincivero et al. 2004), 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 supine position in present study while the optimal angle was often determined when 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 such that the quadriceps muscle group can produce maximal force. Interestingly, these results highlight an inter-individual variability of the slack angle (95% CI ranged from 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 three limitations. First, myoelectrical activity was not recorded to ensure that the muscles remained passive. However, similar to what was done in previous studies (Koo et al. 2014; Lacourpaille et al. 2013), the participant was verbally instructed to stay relaxed before each knee flexion. In addition, an experimenter 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 position. Third, only 9 male rowers were recruited in present study. Although it has been observed gender has no effect on slack angles of muscle heads in triceps surae (Miyamoto, et al. 2018), it should be careful to generate the results to females.

2.5 Conclusion

This is the first study to experimentally determine the muscle mechanical 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 works are needed to determine whether changes in muscle stiffness are muscle-specific in pathological conditions or after interventions such as stretching protocols.

Chapter 3

Age-related alteration in passive and active muscle stiffness in healthy young and senior females

3.1 Abstract

Purpose: The study aimed to investigate the age effect on passive and active stiffness of quadriceps femoris muscles and to explore the relationship between passive muscle stiffness and muscle performance.

Methods: The passive muscle shear modulus of rectus femoris (RF), vastus lateralis (VL), and vastus medialis (VM) in 20 young and 20 senior females was measured at three muscle stretched length by passive flexing the knee joint at 30°, 60°, and 90°. The maximal isometric voluntary contraction (MIVC) of knee extension, six-minute walk test (6MWT), and five times sit-to-stand test (FTSST) were measured. The active muscle stiffness of the three muscles was measured during isometric knee extension at an intensity of 30% MIVC at 30°, 60°, 90° knee flexion.

Results: The passive shear modulus was significantly higher in the senior group than in the young group for the RF and VL muscles at 60° and 90° of knee flexion. The passive shear modulus was significantly increased by 34% ($p = 0.006$) and 56% ($p = 0.000$) for the RF; and by 13% ($p = 0.013$) and 13% ($p = 0.013$) for the VL at 60° and 90° of knee flexion, respectively. The RF passive shear modulus could predict 28.3% of the maximal isometric voluntary contraction torque measured at 60° of knee flexion ($p = 0.017$) across the 2 age groups. The active shear modulus of VM, RF, and VL at 30º knee flexion in senior females was higher than young females by 10%, 20%, and 21%, respectively. The active shear modulus of VM and VL at 90º knee flexion was significantly greater than that of 30º and 60º knee flexion for both groups (all $p < 0.001$). The active shear modulus at 60º knee flexion was greater than that of 30º knee flexion for VM in the young group ($p = 0.045$) and VL in the senior group ($p = 0.041$). For RF muscle, greater active shear modulus was detected at 60º and 90º knee flexion than that at 30º knee flexion ($p = 0.001$, $p < 0.001$ respectively) in the young group and at 60º knee flexion than that at 30º knee flexion ($p = 0.029$) in the senior group. No significant difference in active shear modulus of any muscle was observed in other knee angles (all $p > 0.123$).

Conclusions: The RF and VL muscles in senior females present greater passive stiffness than young females at a stretched length. In addition, the passive muscle stiffness of RF measured at 60° knee flexion predicts the knee extension force. However, significant age effect on active muscle stiffness was only observed at short muscle length with greater active stiffness in senior females. The active muscle stiffness during isometric extension of knee was affected by knee joint angle.

Key Words: passive muscle tension; elastography; ultrasound; quadriceps femoris; peak torque

3.2 Introduction

Aging is associated with loss of muscle force-generating capacity and progressive reduced mobility. Muscle atrophy has been proposed as the main cause of age-related muscle weakness (Candow and Chilibeck 2005; Landers et al. 2001). However, muscle atrophy cannot completely account for the age-induced force decrease because the force decrease is larger than the atrophy sign (Frontera et al. 2000). For example, the force of aged quadriceps femoris muscle was 40% less than the young while the thickness (one of muscle atrophy signs) of muscle only decreased 23% (Ditroilo et al. 2012). Therefore, other factors may be involved, such as a reduction in the number of fast-twitched muscle fibres (Frontera et al. 2000), altered muscle quality (Mota and Stock 2017), and muscle mechanical properties (Ochala et al. 2004).

Aged-related modulation of muscle mechanical properties has been explored in animal and human studies. Greater stiffness of the soleus muscle was observed in old rats compared to young rats (Alnaqeeb et al. 1984; Rosant et al. 2007). In human studies, age-related increase in muscle elastic modulus (an index of muscle stiffness) was observed in the biceps brachialis muscle when assessed in a lengthened but not in a shortened position (Eby et al. 2015). Using qualitative elastography, Saito et al. (2019) also suggested an increased elastic modulus in the rectus femoris and gastrocnemius muscles with aging. However, contrasting results exist with studies reporting either a lower passive muscle elastic modulus in the aged biceps brachii, rectus femoris, vastus intermittent, vastus medialis, hamstrings, and gastrocnemius muscles (Akagi et al. 2015; Alfuraih et al. 2019) or no difference in aged vastus intermittent, tibial anterior, and medial gastrocnemius muscles compared to young muscles (Arda et al. 2011; Domire et al. 2009; Wang et al. 2014).

Note that in most of the studies reporting an absence of age-related difference, muscles were assessed in a shortened position. Age-related modulation in the lower limb muscle mechanical properties might be position-specific, as previously suggested by Eby et al. (2015) on the biceps brachialis. Therefore, evaluation of mechanical properties of individual muscle heads of the quadriceps should be assessed at different muscle lengths to provide a deeper understanding on age-related change in passive muscle mechanical properties. Such information is important as altered muscle mechanical properties may affect muscle force generation ability (Alfuraih et al. 2019).

It has been reported that passive muscle stiffness can be used to detect the change of muscle function, such as muscle damage induced by eccentric exercise (Andonian et al. 2016; Lacourpaille et al. 2017) and fatigue (Akagi et al. 2017; Bouillard et al. 2012). Two recent studies reported an association between passive muscle elastic modulus, muscle force, and daily function (Alfuraih et al. 2019; Saito et al. 2019). Using ultrasound shear wave elastography, Alfuraih et al. (2019) reported that decreased passive muscle stiffness was associated with weaker muscles and worse physical performance (time in get-up-and-go test and numbers in 30-s chair stand test) in subjects spanning from young to age 94. However, in elderly women living in the community-dwelling, Saito et al. (2019) did not observe any relationship between passive RF stiffness and quadriceps strength. It is unknown whether the association between passive muscle mechanical properties and muscle strength is age specific. In this connection, muscle quality and muscle strength are statistically related in the combined (old and young) and the senior but not in the young adults (Mota and Stock 2017). Age-specific evaluation on the relationship between muscle mechanical properties and muscle strength is needed. Since quadriceps femoris muscle is the primary contributor in sit-to-stand movement (Wretenberg and Arborelius 1994), and the knee extension force was corelated with the distance covered in a six-minute walk test (6MWT) (Lord and Menz 2002)—besides the muscle strength—it is necessary to take the 6MWT and sit-to-stand test (FTSST) five times into account when exploring the association between passive muscle stiffness and muscle performance.

Besides passive muscle stiffness, the muscle stiffness during isometric contraction (i.e., active muscle stiffness) has been reported to be affected by age as well. Wang et al. (2017) has explored the effect of age on active passive stiffness of VI. They found the active stiffness of VI in the young group was greater than the senior group. Relations between active muscle stiffness and force output have been studied extensively. During isometric contraction, increase in active muscle stiffness is linearly associated with increment of muscle force for the upper and lower limb muscles (Ates et al. 2015; Bouillard et al. 2011; Sasaki et al. 2014; Yoshitake et al. 2014). Reduction in active muscle stiffness might be one of the causes for the decreased force output of senior muscles than young muscles (Ditroilo et al. 2012; Landers et al. 2001). More importantly, any dissimilar reduction on the muscle heads of the quadriceps femoris, in particularly between the medial (VM) and lateral (VL, RF), might induce an imbalance force on the patellar. This imbalance is an important issue to be explored.

Therefore, the primary aim of this study was to determine the effect of aging on the passive and active shear modulus (an index of stiffness) of the superficial muscle heads of the quadriceps at different lengthened positions. The second aim was to explore the relationship between passive muscle stiffness of the superficial heads of the quadriceps and muscle performance in the young and senior adults. The hypotheses were as follows. Senior females would present with higher passive muscle stiffness and lower active muscle stiffness when the muscle was at a different muscle length. The changes would be muscle-head specific and only occurred with the muscle beyond its slack angle. Second, passive muscle stiffness would be associated with muscle force output and functional performance.

3.3 Methods

3.3.1 Participants

Twenty senior-aged healthy females (age range: 50.0–70.0 years) and twenty young healthy females (age range: 20.1–32.2 years) were recruited through convenience sampling from the local community. The sample size was estimated with G*Power 3.1 software (Kiel University, Germany) with data from the pilot study on 7 participants in each group. Significance level equaled to 0.05, required power equaled to 0.80, with expected group difference in the passive muscle stiffness over slack angles. Totally, 38 participants (n = 19 in each group) were required. Therefore, 40 subjects were recruited. The demographic information (including age, body weight, height, and BMI) is summarized in Table 3.1. All participants were free of knee pain or injury and history of knee surgery. They volunteered for this study and provided informed written consent. This study has been approved by the Human Subject Ethics Subcommittee of Department of Rehabilitation Sciences of Hong Kong Polytechnic University.

Table 3. 1 The demographic information of participates (means ± SD).

Variable	Young	Senior
n	20	20
Age (years)	25.4 ± 2.8	58.4 ± 5.5*
Body weight (kg)	54.3 ± 4.1	53.6 ± 4.6
Height (cm)	161.4 ± 3.7	155.1 ± 4.8*
BMI (kg/m^2)	20.9 ± 1.7	22.3 ± 2.1

*: $p < 0.01$

3.3.2 Passive muscle stretching

After resting for 10 minutes, the participants lied in the supine position on the bed of isokinetic dynamometer (Cybex, Medway, MA, USA) with their hip at neutral position (0° flexion and rotation). The right leg was measured. By adjusting the dynamometer, the knee joint rotation center (lateral femoris condyle) was aligned with the rotation center of the dynamometer. To minimize the potential effect of conditioning, the participants were passively flexed and extended knee joint 5 cycles at slow velocity (5°/s) from 0° to 90° knee flexion by the dynamometer prior to the measurement. Then the knee was positioned at flexion positions of 30°, 60°, and 90° in sequence.

3.3.3 Muscle stiffness measurement

The shear wave elastography of the Aixplorer ultrasound system with a 4–15MHz linear transducer (Axiplorer, Supersonic Imagine, Aixen-Provence, France) was used to measure muscle stiffness (Figure 3. 1). The ultrasound machine was preset in skeletomuscular mode and shear wave elastography interphase. A same well-trained examiner in musculoskeletal ultrasound examination conducted all the measurement. Three superficial heads of quadriceps femoris muscle, VM, RF, and VL, were measured during passively stretching and active contraction. The measurement locations were marked on the skin with a waterproof pen before the test. For VM and VL, it was 1/5 and 1/3 of the distance from the midpoint of medial and lateral patella border to anterior superior iliac spine as the test position, respectively. For RF, it was 1/2 of the distance from anterior superior iliac spine to the midpoint of the superior tip of the patella. The 50mm transducer was held perpendicularly to the skin with generous gel and slight pressure. The transverse images were obtained to confirm the right muscle was measured and then the longitudinal images were captured in videos at 1 Hz, which was the temporal resolution of the current shear wave elastography version and the spatial resolution was 1×1 mm^2. The probe was aligned with either the muscle fiber direction (VL and VM) or the muscle shortening direction (RF). Good images on the B-mode were achieved by slightly moving the transducer when several muscle fibers could be perceived without disconnection through the image (Blazevich et al. 2006).

The capture time for passive and active shear modulus was 5 and 10 seconds, respectively. During each measurement, the square color box was adjusted to the appropriate size as big as possible according to the muscle thickness.

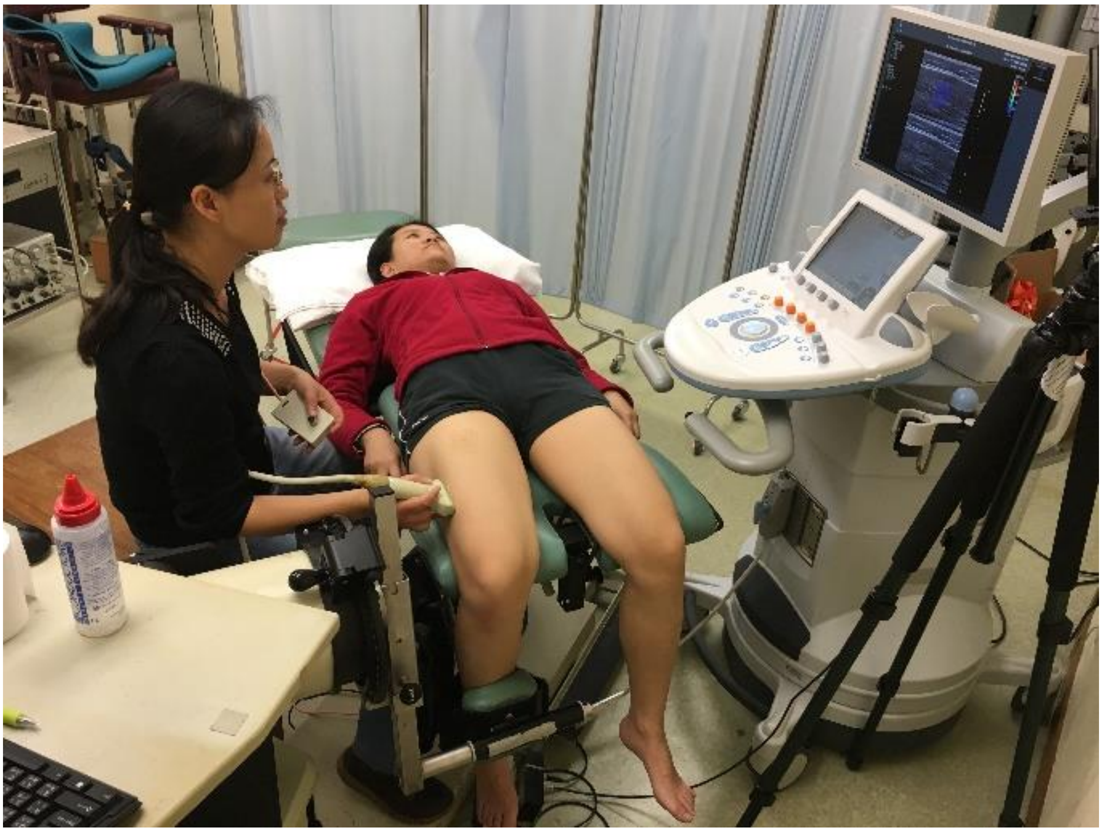

Figure 3. 1 The measurement of the muscle stiffness on the rectus femoris muscle at 90º of knee flexion.

3.3.4 Six-minute walk test (6MWT)

After the passive muscle stiffness measurement was completed, the functional capacity of the participants was measured using six-minute walk test (6MWT), which was valid and reliable for clinical assessment of gait function (Steffen et al. 2002). Briefly speaking, the test was conducted in a 15-meter-long corridor. After resting for 10 minutes, the participant was instructed to walk on a flat, hard surface as far as possible in 6 minutes. Standardized oral encouragement of “You are doing well” and “Keep up the good work” was given to the participants at 1, 3, 5 minutes and at 2, 4 minutes respectively during the walk together with the mention of the time left. The total distance in meters the participants covered were recorded as the performance.

3.3.5 Five times sit-to-stand test (FTSST)

Doing the sit-to-stand test (FTSST) five times was employed to evaluate the time participants take to rise from a chair and sit down for 5 cycles. The FTSST has demonstrated good reliability in measuring the physical function of lower limbs (Bohannon 2011). The test was conducted using an armless straight back chair with a solid seat. The seat height was 43 cm. The participant sat on the chair and was instructed to fold his or her arms across the chest to standardize arm position first. Then the participant was asked to perform 5 repetitions of rising from the chair to full standing position and sitting down as quickly as possible. The time required to finish the activities was recorded as the score. The endpoint of the time record was the moment when the participant sat with his or her back to the chair after the fifth repetition.

3.3.6 Maximal isometric voluntary contraction (MIVC) test

The participants were positioned supine with their hip at 0° and neutral rotation, the pelvis, trunk, and thigh stabilized with straps. The rotation center of the Cybex ergometer was aligned with the knee joint rotation center (lateral femoris condyle). Prior to measurement, the participants were instructed to perform two submaximal contractions to warm up and become familiar with the test procedure. Each participant conducted three 5-second maximal isometric voluntary contractions with 2-minute interval between each contraction at three knee angles (30°, 60°, and 90° of knee flexion; 0° = full extension). Measurements were conducted at multiple angles corresponding to the common daily tasks, such as walking, climbing stairs, and standing from a seated position. The test was conducted in sequence from 30°, 60°, and 90° with 2-minute rest between each angle. The maximum peak torque of the 3 contractions captured at each angle was recorded as MIVC (N·m).

3.3.7 Test protocol

First the passive muscle shear modulus was measured. For each stretch position, the knee joint was stabilized at the position for 30 seconds before the measurement started in order to make muscles adapt to stretch. Oral instruction was given to participants to keep the muscle relaxed before each capture of image. Five successive images were captured for further management in video format. Then the participants underwent functional tests of 6MWT and FTSST.

After the above measurements were finished, the participants underwent MIVC evaluation, which was followed by active muscle shear modulus measurement. The test sequence of muscle was randomized both in passive and active muscle stiffness measurement. The active shear modulus of VM, RF and VL muscles was measured at the same positions as passive shear modulus, that is at 30°, 60°, and 90° knee flexion in sequence. The active shear modulus of one muscle was measured when the participants performed one isometric contraction of knee extension at intensity of 30% MIVC. Between each contraction, a 2-minute rest was given.

To assess the within-day, the passive and active muscle shear modulus of 10 participants (8 senior and 2 young) was measured twice within the same session by means that the transducer was taken away and relocated the measured site between the two measurements. To assess the between-day reliability, the same measurement was conducted at the same time the next day.

3.3.8 Data analysis

The shear wave elastography data were exported in mp4 format and sequenced in png. Image processing was performed using a custom Matlab script (MathWorks, Natick, MA). Each image was carefully inspected for artefacts. If artefacts were present in any image, the region of interest (ROI) was reduced in size to remove the artefact from all images within that stretching condition. The coloured 2-D maps of shear moduli were converted into shear modulus values (the index of muscle stiffness) and the shear moduli were averaged for further analysis.

Statistical analysis was conducted using 21.0 SPSS software package (New York, USA). Statistical significance level of p value was set to be less than 0.05. All variables were checked for normality by the Shapiro–Wilk test. The intraclass correlation coefficient (ICC) was calculated to determine reliability of passive and active shear modulus. Using previously established criteria, reliability was presumed to be good if ICC > 0.75, moderate if ICC was 0.50-0.75, and poor if ICC < 0.50 (Portney and Watkins 2009). Independent sample t-test was performed to compare the difference of basic demographic information, MIVC at each angle, 6MWT and FTSST between young and senior groups. Three-way mixed measures ANOVA (within subject factors: muscle and knee angle; between subject factor: age (young and senior)) were used to compare the passive and active muscle stiffness of the three muscles. Post-hoc analyses were performed when appropriate using the Bonferroni method. Possible relationships between shear modulus and muscle strength and muscle functions were assessed using the Pearson correlation coefficient or Spearman's rank correlation coefficient in the young and senior groups, as well as in the combined group. Linear regression was performed to assess the independent relationship when significant correlation was detected.

3.4 Results

All the participants completed the measurement and none of them complained of any pain or other discomfort.

3.4.1 The reliability of the passive and active shear modulus

Table 3.2 shows the within-day reliability and the between-day reliability of the passive and active muscle shear modulus for the three muscle heads at different joint angles. In general, for passive shear modulus good within-day reliability with ICC of 0.877 to 0.979 of the 3 tested muscle heads at all tested angles were detected. The between-day reliability was moderate to good with ICC ranging from 0.689 to 0.918 of the 3 tested muscle heads at all tested angles. For active shear modulus, good within-day and between-day reliability was also achieved with ICC range from 0.836 to 0.980 and 0.793 to 0.980, respectively.

3.4.2 Age-related modulation on passive muscle shear modulus

The shear modulus values of the three muscles are depicted in Table 3.3 and Figure 3.1. There was a significant main effect of group, muscle, and angle, and a significant interaction between muscle and angle and group (all p values < 0.001). *Post hoc* analysis revealed that the muscle shear modulus of RF and VL was significantly higher in the senior than the young group when the knee was positioned at 60° and 90° of knee flexion (Table 3.3). Specifically, significant increase of 34% ($p = 0.006$) and 13% ($p = 0.031$) in the RF and VL, respectively, were observed in the senior than the young group when the knee was positioned at 60° of knee flexion. At 90° knee flexion, the increase was 56% ($p = 0.000$) and 13% ($p = 0.031$) for RF and VL, respectively. When considering the VM muscle, no significant difference in shear modulus was observed between groups (all p values > 0.05).

Table 3. 2 The ICC values of within-day and between-day reliability for passive and active shear modulus.

	Passive shear modulus			Active shear modulus		
Knee angles	VM	RF	VL	VM	RF	VL
Within-day reliability						
30°	0.877	0.979	0.944	0.955	0.836	0.867
60°	0.940	0.963	0.921	0.898	0.980	0.907
90°	0.928	0.965	0.973	0.951	0.946	0.942
Between-day reliability						
30°	0.910	0.851	0.891	0.970	0.793	0.838
60°	0.781	0.791	0.907	0.908	0.934	0.912
90°	0.689	0.918	0.735	0.980	0.897	0.922

VM: vastus medialis; RF: rectus femoris; VL: vastus lateralis.

Table 3. 3 Comparison between the young and senior adults on passive shear modulus of the superficial heads of the quadriceps muscle.

Muscles	Young (kPa)	Senior (kPa)	Group difference (kPa)	P-value	95% CI
VM					
30°	3.71 ± 0.36	3.70 ± 0.59	-0.02 ± 0.77	0.918	-0.30 - 0.33
60°	5.31 ± 0.75	5.10 ± 0.98	-0.22 ± 1.52	0.438	-0.34 - 0.77
90°	8.17 ± 1.40	8.70 ± 1.40	0.53 ± 1.84	0.173	-1.30 - 0.24
RF					
30°	4.85 ± 1.35	4.85 ± 1.51	0.00 ± 1.67	0.998	-0.91 - 0.92
60°	7.46 ± 1.90	10.03 ± 3.42	2.57 ± 3.57	0.006	-4.36 - -0.78
90°	15.46 ± 4.12	24.10 ± 5.17	8.64 ± 5.54	0.000	-11.63 - -5.64
VL					
30°	4.22 ± 0.71	4.72 ± 1.42	0.50 ± 1.47	0.172	-1.22 - 0.23
60°	5.47 ± 0.71	6.35 ± 1.58	0.88 ± 1.68	0.031	-1.67 - -0.09
90°	7.96 ± 1.18	9.03 ± 1.77	1.07 ± 2.08	0.031	-2.04 - - 0.10

VM: vastus medialis; RF: rectus femoris; VL: vastus lateralis.

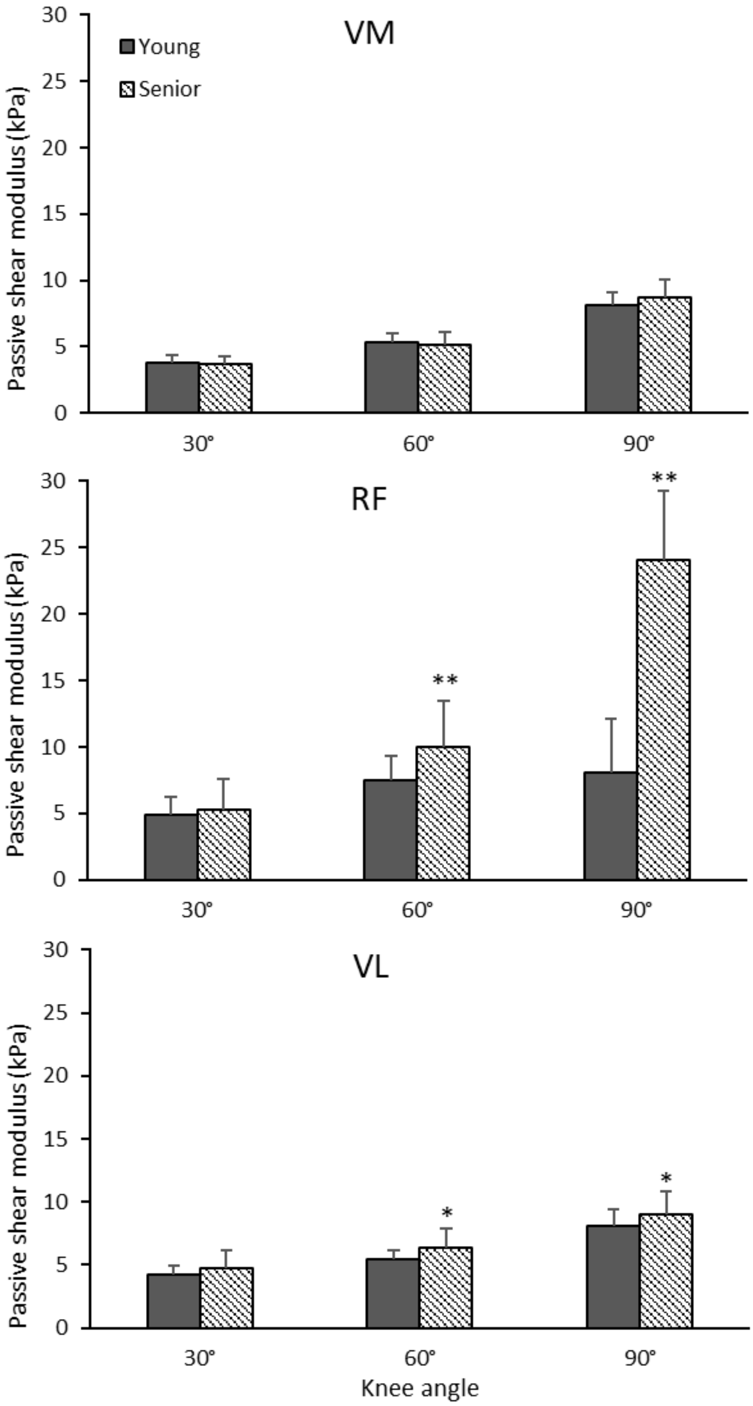

Figure 3. 2 The passive shear modulus of VM, RF, and VL muscles of the young and senior groups at different knee angles. Compared with the young group, the senior group exhibited significantly higher shear modulus values when assessed beyond 60° of knee flexion for the RF and VL muscles. * $p < 0.05$, ** $p < 0.01$. VM: vastus medialis; RF: rectus femoris; VL: vastus lateralis.

3.4.3 Age-related modulation on active muscle shear modulus

The active shear modulus of the VM, RF, and VL muscles is shown in Table 3.4. According to the two-way ANOVA analysis, there was a significant main effect of group on active shear modulus ($p = 0.035$) without muscle effect ($p = 0.350$) and any interaction effect between muscle and group at 30º knee flexion ($p = 0.535$) (Figure 3.3). The active shear modulus at 30º knee flexion in the senior group was higher than the young group by 10%, 20%, and 21% for VM, RF, and VL, respectively. No significant main effect of group, muscle, and interaction effect between muscle and group was observed at 60º and 90º knee flexion (all $p > 0.091$).

Figure 3.4 shows the comparison of active shear modulus at different angles for the two groups. The active shear modulus of VM and VL at 90º knee flexion was significantly greater than that of 30º and 60º knee flexion for both young and senior groups (all $p < 0.001$). The active shear modulus at 60º knee flexion was greater than that of 30º knee flexion for VM in young group ($p = 0.045$) and VL in senior group ($p = 0.041$). For RF muscle, greater active shear modulus was detected at 60º and 90º knee flexion than that at 30º knee flexion ($p = 0.001$, $p < 0.001$ respectively) in young group and at 60º knee flexion than that at 30º knee flexion ($p = 0.029$) in the senior group. No significant difference in active shear modulus of any muscle was observed in other knee angles (all $p > 0.123$).

Table 3. 4 The active shear modulus of the VM, RF, and VL muscles when the loading was 30% MIVC at three knee angles.

Knee angle	Group	Active shear modulus (kPa)		
		VM	RF	VL
30°	Young	36.23 ± 11.28	47.84 ± 9.01	29.60 ± 14.05
	Senior	38.65 ± 14.78	55.05 ± 15.23	33.48 ± 10.86
60°	Young	41.80 ± 17.44	60.85 ± 15.37	31.00 ± 8.53
	Senior	38.95 ± 13.19	64.58 ± 21.06	39.16 ± 12.05
90°	Young	79.04 ± 24.44	67.62 ± 20.29	70.42 ± 16.09
	Senior	70.42 ± 27.00	59.46 ± 30.68	71.24 ± 21.03

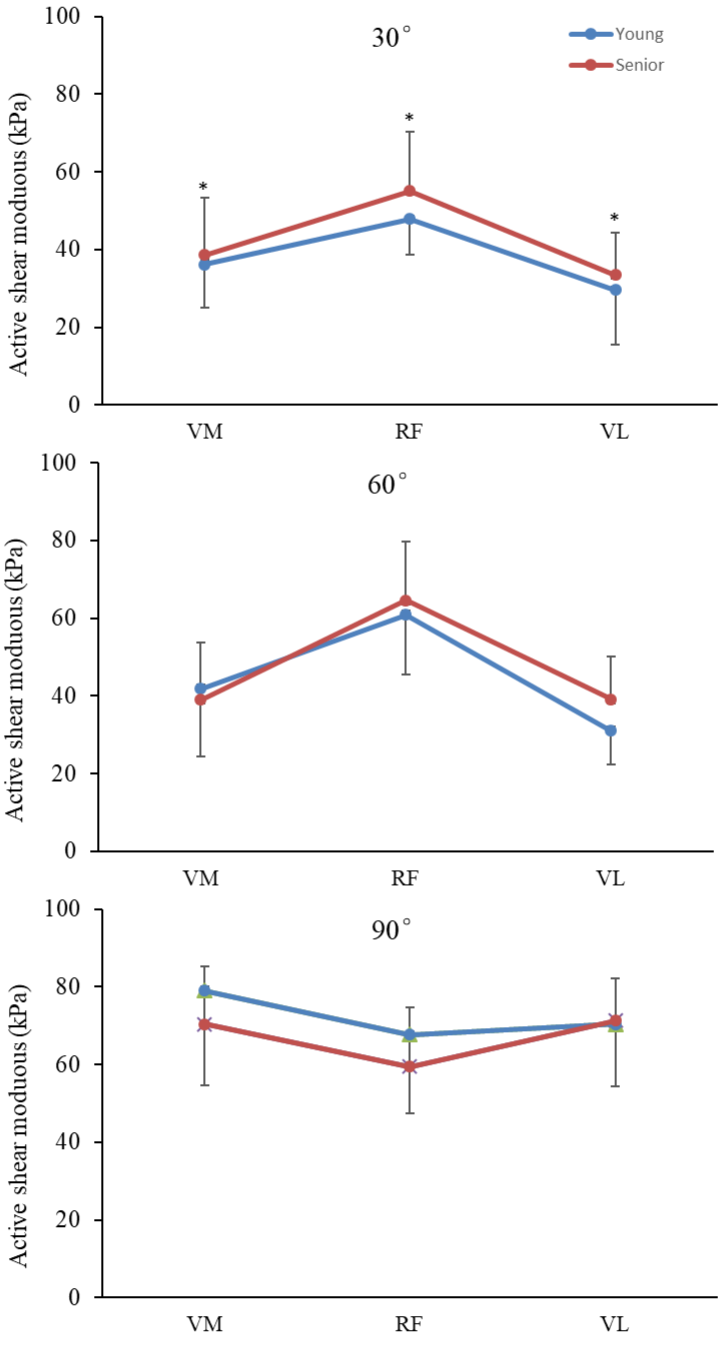

Figure 3. 3 The comparison of active shear modulus of VM, RF, and VL muscles between groups. The active shear modulus of the three muscles was significantly greater in the senior group than the young group when the MIVC was used as covariable at 30º knee flexion. * $p < 0.05$. VM: vastus medialis; RF: rectus femoris; VL: vastus lateralis.

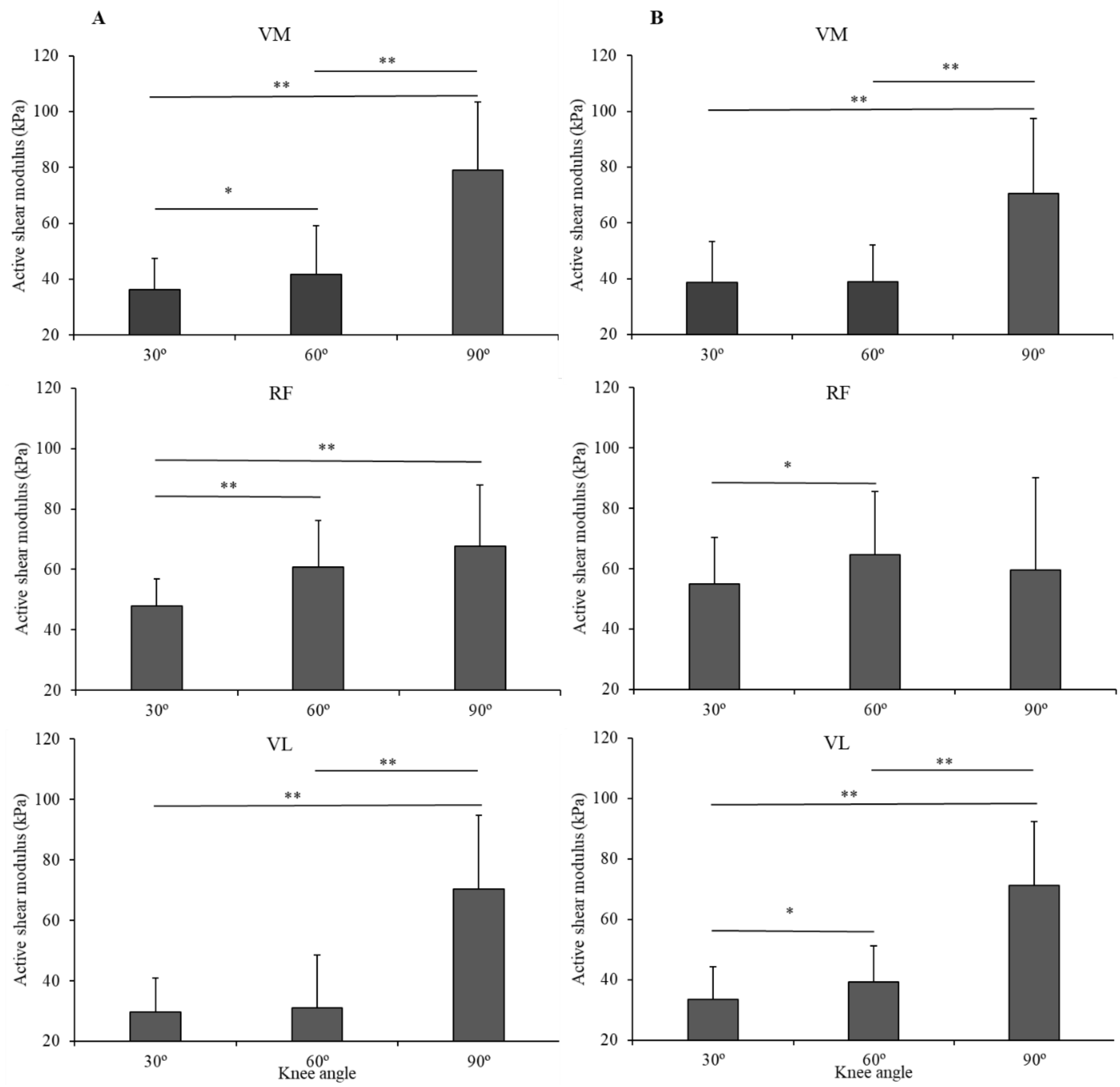

Figure 3. 4 The angle effect on active shear moduli among VM, RF, and VL muscles. A) young group; B) senior group. * p < 0.05, ** p < 0.01. VM: vastus medialis; RF: rectus femoris; VL: vastus lateralis.

3.4.4 The muscle performance

The results of MIVC, 6MWT, and FTSST of the two groups are depicted in Table 3.5. MIVC was significantly lower in the senior group than the young group when the leg was positioned at 30° ($p = 0.044$), 60° ($p = 0.003$), and 90° ($p < 0.001$) of knee flexion. Compared with the young group, the senior group covered a significantly shorter distance when performing 6MWT (-14.0%, $p < 0.001$) and needed significantly longer time to finish FTSST (+29.1%, $p < 0.001$).

Table 3. 5 The comparison of MIVC, 6MWT, and FTSST between young and senior groups.

	Young	Senior	p value
MIVC at 30° (N·m)	46.15 ± 9.46	40.40 ± 7.93	0.044
MIVC at 60° (N·m)	82.40 ± 18.50	65.55 ± 15.52	0.003
MIVC at 90° (N·m)	95.20 ± 22.50	64.15 ± 16.86	< 0.001
6MWT (m)	558.1 ± 43.8	480.0 ± 49.9	< 0.001
FTSST (s)	7.23 ± 1.26	9.36 ± 1.80	< 0.001

MIVC: maximal isometric voluntary contraction; 6MWT: six-minute walk test; FTSST: five times sit-to-stand test.

3.4.5 Relationship between passive muscle stiffness, muscle strength, and function

Due to the values of FSTST was not normal distribution (Kolmogorov-Smirnova $p = 0.035$) and the values of MIVC and 6MWT were different between young and senior groups although the combined data were normal distribution, Spearman correlation coefficients was used to do the relationship analysis between passive shear modulus and muscle strength. The results are depicted in Table 3.6. The passive RF shear modulus at 60° knee flexion was moderately and negatively correlated with muscle strength in the combined (rho = -0.504, p = 0.001) and senior group (rho = -0.485, p = 0.030) and had a trend of correlation in the young group (rho = -0.395, p = 0.085) (Table 4.6, Figure 3.4). The passive RF shear modulus at 90° knee flexion was moderately and negatively correlated with muscle strength in the combined group (rho = -0.447, p = 0.004) (Table 3.6). Linear regression model was conducted with age group and muscle shear modulus entered as predictive factors. At 60º of knee flexion, the RF shear modulus was a significant predictor of muscle strength (adjusted $r^2 = 0.283$, $p = 0.017$); at 90º of knee flexion, age group was the only predictor of muscle strength (adjusted $r^2 = 0.362$, $p < 0.003$). There is no significant relationship between passive shear modulus of all the muscles and 6MWT and FTSST in both groups except shear modulus of RF at 30º of knee flexion in young group that was associated with FTSST (Table 3.7).

Table 3. 6 Correlation coefficients among passive muscle shear modulus of the superficial heads of the quadriceps and MIVC.

Torque/ shear modulus		Young			Senior			Combined		
		30º	60º	90º	30º	60º	90º	30º	60º	90º
VM	30º	-0.160			0.163			0.006		
	60º		0.350			-0.091			0.180	
	90º			-0.186			0.024			-0.212
RF	30º	-0.052			-0.008			-0.110		
	60º		-0.395			-0.485*			-0.504**	
	90º			-0.058			-0.117			-0.447**
VL	30º	0.157			-0.166			-0.085		
	60º		-0.015			-0.280			-0.236	
	90º			0.102			-0.297			-0.272

*: $p < 0.05$, **: $p < 0.01$. VM: vastus medialis; RF: rectus femoris; VL: vastus lateralis.

Table 3. 7 Correlation coefficients (p) among passive muscle shear modulus of the superficial heads of the quadriceps and functional performance.

		Young		Senior	
		6MWT	FTSST	6MWT	FTSST
VM	30º	-0.264(0.261)	-0.126(0.595)	0.145(0.541)	0.420(0.065)
	60º	-0.201(0.396)	-0.063(0.791)	0.294(0.208)	0.115(0.631)
	90º	-0.038(0.872)	0.005(0.982)	0.323(0.165)	0.191(0.421)
RF	30º	-0.098(0.682)	0.518(0.019)	0.074(0.757)	0.089(0.709)
	60º	0.065(0.786)	0.424(0.062)	0.264(0.260)	0.234(0.322)
	90º	-0.334(0.150)	0.196(0.408)	0.285(0.223)	0.159(0.503)
VL	30º	0.172(0.468)	0.249(0.290)	0.189(0.425)	-0.018(0.940)
	60º	0.146(0.539)	0.299(0.200)	0.193(0.414)	0.051(0.830)
	90º	-0.327(0.159)	-0.094(0.693)	-0.017(0.945)	0.015(0.950)

6MWT: six-minute walk test; FTSST: five times sit-to-stand test. VM: vastus medialis; RF: rectus femoris; VL: vastus lateralis.

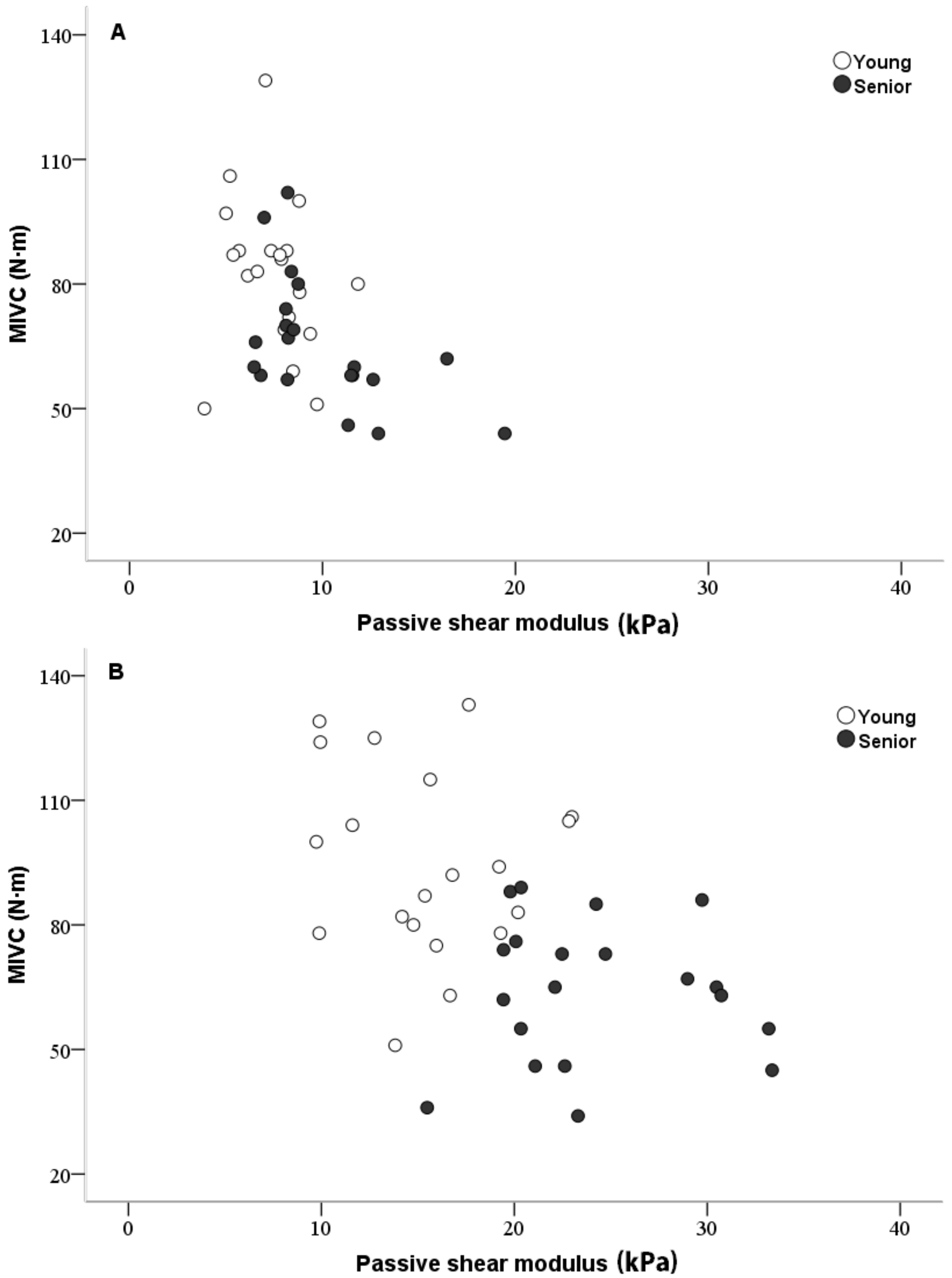

Figure 3. 5 The scatterplots between passive shear modulus of rectus femoris muscle and the peak torque of maximal isometric voluntary contraction at (A) 60° and (B) 90° of knee flexion. MIVC denotes maximal isometric voluntary contraction.

3.5 Discussion

The present study indicated age effect on passive and active muscle stiffness. The aging effect is angle and muscle-head specific. A higher RF and VL passive stiffness beyond the slack angle was observed in the senior group more than young females. More important, higher passive RF stiffness at 60º knee flexion was associated with lower force output. The active shear modulus of VM, RF, and VL at 30º knee flexion in senior females was higher than young females.

Before the main study, reliability of shear wave elastography was assessed. The results demonstrated good within and between-day reliability in passive stiffness measurement of quadriceps femoris muscle. The reliability is consistent with numerous previous studies that have established good reliability in a wide range of muscles (Hatta et al. 2015; Jeon et al. 2018; Lacourpaille et al. 2012; Leong et al. 2013; Nordez and Hug 2010). Even the interval time ranged from 2 days to even 3 weeks; the between-day reliability was still good (Cortez et al. 2016; Lacourpaille et al. 2012). While muscles performed isometric contraction, the within and between-day reliability of active muscle stiffness measurement was pretty good as well. The ICCs of within-day reliability in the present study ranged from 0.836 to 0.980, which were very similar to reliability estimated in other contracted muscles, such as upper trapezius muscle (Leong et al. 2013), gastrocnemius medial muscle (Jeon et al. 2018), supraspinatus and deltoid muscles (Kim et al. 2018). Limited study has examined and reported on between-day reliability. To our knowledge, only Koppenhaver et al. (2018) reported moderate between-day reliability in contracted lumbar erector spinae and multifidus muscles with lower ICCs (0.44 to 0.66) than present study with ICCs from 0.793 to 0.980. The relatively low between-day reliability of active muscle stiffness in lumbar muscles is possibly due to the deep position under thick and dense thoracolumbar fascia, which might decrease the stability of elastography images (Moreau et al. 2016). Taken together, shear wave elastography provides good test–retest measurements on passive and active muscle stiffness on the superficial heads of the quadriceps femoris muscle.

Using shear wave elastography, the present study revealed an age-related increase in the passive muscle stiffness of VL and RF muscles that was length specific. In addition, the passive stiffness of RF is a significant predictor of muscle strength in females. The present results echoed with previous findings that passive stiffness of aged muscles is higher than that of young muscles either in animals (Rosant et al. 2007; Stearns-Reider et al. 2017) or humans (Ditroilo et al. 2012; Eby et al. 2015).

Meyer and Lieber (2011) suggested that the extracellular matrix (ECM) is largely responsible for the muscle stiffness. In this way, animal experiments showed that muscle fiber bundles, which contain ECM, displayed almost a six times higher shear modulus than individual muscle fibers and muscle fiber groups, which do not contain ECM (Meyer and Lieber 2011). Experiments on human tissues revealed that the stiffness of muscle bundles is 16-fold than that of single muscle fibers, despite that the ECM only accounts for about 5% of the area muscle bundles (Lieber et al. 2003). When muscle bundles of aged and young mice were measured, the shear modulus of aged muscles was much higher than that of adult muscles. However, when the ECM was removed from the single muscle fibers of aged and adult muscles, such difference disappeared (Wood et al. 2014). Taken together, these results suggest that an age-related increase in muscle shear modulus might be associated with age-related changes in the ECM. In this connection, the amount of ECM increases with aging (Wood et al. 2014). The collagen, which is the primary component of ECM, was observed to be increasingly deposited in aged skeletal muscle (Lacraz et al. 2015). In this latter animal experiment, the accumulated ECM induced a nearly 3 times increase in stiffness of quadriceps femoris muscle of aged muscle in animal (Lacraz et al. 2015). Also, histological analysis revealed that the amount of a highly compliant element in ECM such as elastin and collagen type III was lower in aged muscles than in young muscles.

Aside from ECM, changes in the percentage of muscle fibre type associated with aging might also lead to an increase in muscle stiffness in older adults. Lee et al. (2006) observed an increase in area and number percentage of Type I muscle fibres with age and type I muscle fibre in response to passive stretch demonstrated higher stiffness than type II fibers (Ramsey et al. 2010). In addition, the retrogression of metabolism may also contribute to increased stiffness of aged muscles. It is supported by the accumulation of advanced glycation end products in collagenous tissues and 2-folded increased pentosidine in old muscles (Haus et al. 2007). The exact underlying mechanisms responsible for the observed increased shear modulus in a senior population might be multi-factorial and need to be further investigated.

Findings from this study indicate that an age-related increase in passive shear modulus is position specific. More specifically, the passive shear modulus of RF and VL muscles was significantly greater in the senior group than young adults when the muscle heads were positioned at and beyond 60º of knee flexion. In this connection, muscle length adjusted by joint position has been demonstrated to affect passive muscle stiffness (Hug et al. 2013; Koo et al. 2014; Xu et al. 2018). When muscle is stretched beyond the slack length, the muscle fiber will become taut and even reach to a cross-bridges detached length (Proske and Morgan 1999). In animal study, age-related increase in passive muscle stiffness was detected when the muscles were assessed in their lengthened but not in their shortened position (Rosant et al. 2007). Similar findings were reported in the upper limb muscle (Ditroilo et al. 2012; Eby et al. 2015). The findings showed age-related difference only when the knee was positioned above 60° of flexion in accordance with these previous works. Overall, it might partially explain why no significant difference was detected between the young and elderly populations when the muscles were assessed in their shortened position (Arda et al. 2011; Domire et al. 2009; Wang et al. 2014). However, the age-related increase in passive shear modulus was not observed in VM. Considering the pennation angle of RF and VL is lower than VM (Arnold et al. 2010), the age effect on passive shear modulus which occurred on RF and VL but not VM might be explained.

When the knee was positioned at 60º of knee flexion, RF shear modulus explained 28.3% of the variance in maximal isometric extension torque. At 90º of knee flexion, age group was the only predictor. That is, the larger the RF shear modulus, the lower the knee extensor strength when the knee was positioned at its mid-range (i.e., 60º of knee flexion). There is only one study that reported relationships between muscle strength and muscle stiffness in subjects throughout adult age span (Alfuraih et al. 2019). Interestingly, Alfuraih and his colleague (2019) found that reduced muscle stiffness of the muscle heads of the quadriceps was associated with lowered maximal quadriceps strength. The authors recruited subjects of both genders, and quantified peak isokinetic knee extension torques. More important, the RF shear modulus was measured at its shortened positions (with the hip and knee at 0º and 90º of flexion).

Similar to the present study, Saito et al. (2019) observed a negative association between the RF shear modulus and quadriceps maximal strength in older females (ρ = -0.302) despite the correlation was not statistically significant. Aside from reduced muscle size (Candow and Chilibeck 2005; Landers et al. 2001), lowered muscle quality is also associated with lowered isometric strength (Cadore et al. 2012; Fukumoto et al. 2012; Rech et al. 2014). The present study illustrates an association between muscle stiffness and muscle function in healthy subjects, ages ranging between 20 and 70. Such relationship could not be detected when the knee was positioned at 90º of flexion. This is possibly because at 90º knee flexion, the muscle is located at the descending limb of the length-tension curve at which the relationship between muscle stiffness and muscle force has been changed (Ichinose et al. 1997; Marginson and Eston 2001; Pincivero et al. 2004).

Contrast to a greater passive muscle stiffness in the senior group at stretched muscle length, the significant age effect on active muscle stiffness was only observed at short muscle length in present study. Contrary to the hypothesis, the active stiffness of VM, RF, and VL in senior females was greater than young females at 30º of knee flexion, which was inconsistent with early studies. It has been reported that the active stiffness of VM in the young group was significantly greater than the senior group during 20% isokinetic knee extension at 30º knee flexion (Debernard et al. 2011). The greater active stiffness of VI muscle (another head of quadriceps femoris muscle) was also reported in young adults than senior adults (Wang et al. 2014; Wang et al. 2017). In these studies, the force deceases with aging was not considered as a covariable to affect the active muscle stiffness during group comparison. In addition, the different joint position to measure active shear modulus may be a cause of the different result. The age effect on active stiffness was observed at 60º and 90º knee flexion with participants in a seated position (Wang et al. 2014; Wang et al. 2017). While in present study, the variance was detected at 30º knee flexion with participants in supine position. Another possible explanation for the variance could be that the measured muscle was the superficial heads (RF, VL, VM) in this study but the deep head (VI) in Wang studies (Wang et al. 2014; Wang et al. 2017).

The angle effect on active stiffness of VM and VL was consistent with an early study that demonstrated VI muscle presented larger active muscle stiffness at 90° knee flexion than at 60° (Wang et al. 2017). However, the active stiffness of RF at 90° knee flexion was not greater than 60° in both groups. This may be a result from different hip positions they adopted with participants siting with their hips at around 90º in the study of Wang et al. (2017) while at 0º in present study. However, the active stiffness VM and VL was not affected by hip position. In addition, the active muscle stiffness was not measured at 30º knee flexion (Wang et al. 2017). From the present study, it seems the active stiffness did not increase much with the knee angle flexed from 30º to 60º for VM and VL, although VM in the young group and that of VL in the senior group presented statistically greater active stiffness at 60º than 30º knee flexion (p = 0.045 and 0.041, respectively).

It has been reported that the optimal angle of quadriceps femoris muscle at which the knee extensor acquired peak torque was 70–80° knee flexion (Ichinose et al. 1997; Marginson and Eston 2001; Pincivero et al. 2004). When the knee is positioned at 90° knee flexion, the VM and VL muscles are located at the descending limb of the length-force curve at which the muscle force is lower than that at an optimal angle. As greater active muscle stiffness was beneficial to muscle force generation (Kalkhoven and Watsford 2018), the increased active stiffness may act as a compensation to muscle performance. EMG activity of VL at 90º knee flexion, which was greater than other angles when the knee extended isometrically and maximally (Suter and Herzog 1997); this may be an evidence of this kind of compensation. However, VM and VL appeared to have a similar active stiffness at 30º and 60° knee flexion. This may suggest that no compensation is needed when the muscle contracted at the ascending limb of length-force curve. In regard to comparable active stiffness of RF at 60º and 90º knee flexion, the possible reason may be related to its biarticular structure and smallest cross-sectional area accompanied by the least force output among the four heads of quadriceps (Arnold et al. 2010). Further research is needed to prove the supposition.

The limitation of this study should be considered. First, for reliability measurement, the small sample size of 10 participants had the risk of increase the reliability. Another limitation of the present study was that the participants were not real aged people as their ages ranged from 50 to 70 years old. This age group was chosen because atrophy apparently almost occurred in all the skeletal muscles by 50 years old (Lexell et al. 1988). It has been reported that muscle shear modulus increased with age in person more than 60 years old (Eby et al. 2015). If more participants over age 65 were measured, it was speculated that the age effect on increased passive muscle stiffness would be more noticeable. The other limitation was that only females were recruited in this study, which was based on the findings that no difference in passive stiffness was observed between males and females (Akagi et al. 2015; Alfuraih et al. 2019; Botanlioglu et al. 2013). However, in the next chapter, it was found that the passive stiffness of RF muscle in males was greater than females when the knee was passively flexed to a long length. For active muscle stiffness, females showed greater active stiffness than males during isometric contractions, which was a conflict with the previous study (Wang et al. 2017). Therefore, the conclusion should not be extended to males.

3.6 Conclusion

In conclusion, the results showed that the rectus femoris and vastus lateralis muscles in senior females present greater passive stiffness than young females at a stretched length. In addition, the passive muscle stiffness of RF measured at 60° knee flexion predicts the knee extension force. However, significant age effect on active muscle stiffness was only observed at short muscle length with greater active stiffness in senior females. The active muscle stiffness during isometric knee extension was affected by the knee joint angle.

Chapter 4

Gender effect on passive and active stiffness of individual quadriceps femoris muscle

4.1 Abstract

Purpose: The study aimed to compare the gender effect on passive stiffness of quadriceps femoris muscle in adults when the muscle was passively stretched to difference length and active stiffness when the knee isometrically extended at same intensity.

Methods: The passive muscle shear modulus of rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM) in 10 young males and 20 young females was measured at three muscle length by passive flexing the knee joint at 30°, 60°, and 90°. The maximal isometric voluntary contraction (MIVC) of knee extension at 30°, 60°, and 90° knee flexion was measured. The active muscle stiffness of the three muscles was measured during isometric knee extension at an intensity of 30% MIVC at 30°, 60°, 90° knee flexion.

Results: The passive shear modulus of RF muscle in males was significantly greater at 60º ($p = 0.019$) and 90º ($p = 0.017$) knee angles than females. No significant difference was found for the passive shear modulus of VM and VL muscle ($p > 0.05$). The MIVC of males was significantly greater than females at all three angles. The active shear modulus of the three muscles was higher in females than males at 60º and 90 º knee flexion ($p = 0.024$ and $p < 0.001$ respectively).

Conclusions: The passive stiffness of RF muscle in males was greater than females when the muscle was passively stretched to a long length. However, no gender difference was observed in VM and VL muscles. The active muscle stiffness of VM, VL, and RF muscles was higher in females than males at long muscle length.

Key Words: passive muscle tension; elastography; active muscle tension, ultrasound; quadriceps femoris; peak torque

4.2 Introduction

Muscle stiffness is physiologically critical for muscle to execute its contractile function (Sasaki et al. 2014; Whittington et al. 2008). Passive and active muscle stiffness represents stiffness of the muscle during its relaxed or contractile status, respectively. Gender differences, muscle morphology, and architecture might induce differences on muscle stiffness at rest and during contraction.

Using ultrasound elastography, several studies have revealed that males presented significantly higher passive stiffness in gastrocnemius and masseter muscles than females (Arda et al. 2011; Yoshida et al. 2017). On the contrary, for biceps brachii muscle, there was a trend of higher passive stiffness in females than males, but the authors did not report any significant difference (Eby et al. 2015). Apart from the different comparisons, another study carried out by Brandenburg et al. (2015) showed there is no gender difference of passive muscle stiffness on lateral gastrocnemius muscle. Therefore, the effect of gender on passive muscle stiffness needs further study.

Some attention has been paid to differences of active muscle stiffness across genders. For example, when muscles contracted isometrically, males presented higher active stiffness than females for hamstring muscles (Blackburn et al. 2004), and 27– 43% higher for quadriceps femoris muscle (Granata et al. 2002b). During functional activity, such as functional hopping, similar results were observed as well (Granata et al. 2002a). When using shear modulus to measure active muscle stiffness directly, Botanlioglu et al. (2013) similarly demonstrated the active muscle stiffness of males was higher than that of females, which was accompanied by larger force produce while the participants performed maximal isometric knee extension. But it should be noted that the higher active stiffness might depend on greater force output of males. Thus, whether the active muscle stiffness of males is greater than females when the contracting intensity was standardized is still unclear.

Therefore, the purpose of this study was to compare the gender effect on passive stiffness of individual heads of quadriceps femoris muscle when the muscle was passively stretched to a different length and active stiffness when the knee isometrically extended at the same intensity. it was hypothesized that passive stiffness would be greater in males than females at long muscle length, and males would be stiffer than females in isometric contraction.

4.3 Methods

4.3.1 Participants

Thirty (20 females and 10 males) young healthy adults were recruited in this study. Their age, body mass, and height were 26.5 ± 4.2 years old, 64.2 ± 8.1 kg, 171 ± 5 cm for males and 24.4 ± 2.8 years old, 54.3 ± 4.1 kg, 161 ± 4 cm for females, respectively. None of the participants suffered known musculoskeletal disorder or injuries in their legs. All the participants provided informed written consent before the study. This study has been approved by the local Human Subject Ethics Subcommittee (Department of Rehabilitation Sciences, Hong Kong Polytechnic University).

4.3.2 Passive muscle stretching

The participants lied in the supine position on the bed of isokinetic dynamometer (Cybex, Medway, MA, USA) with hips at neutral position (0° flexion and rotation). The right leg was measured. The lateral femoris condyle, which is the skin mark for rotation center of knee joint, was kept in line with the rotation center of the dynamometer through adjusting the dynamometer. To minimize the potential effect of conditioning, the knee joint was passively flexed and extended by the dynamometer five times at a slow angular velocity (5°/s) from 0° to 105° knee flexion prior to the measurement. Then the knee was placed at 30°, 60°, and 90° flexion positions sequentially for passive stiffness measurement.

4.3.3 Muscle stiffness measurement

The passive and active muscle stiffness was assessed by the shear wave elastography of the Aixplorer ultrasound system (Axiplorer; Supersonic Imagine, Aixen-Provence, France). All the elastographies were collected by a 4-15MHz linear transducer with a length of 50mm. The ultrasound machine was preset in skeletomuscular mode and shear wave elastography interphase. The same well-trained examiner in musculoskeletal ultrasound examination conducted all the measurements. Three muscles, VM, RF, and VL, were measured during passively stretching and isometrically active contraction. The measurement sites were marked on the skin with a waterproof pen prior to the test. For VM and VL, the distal locations at one fifth and one third of the distance from the midpoint of medial and lateral patella border to anterior superior iliac spine were measured, respectively. For RF, midpoint of the distance from anterior superior iliac spine to the midpoint of the superior tip of the patella was measured. The transducer was held perpendicularly to the skin with enough gel and slight pressure. The transverse images were viewed first to verify the right muscle was measured and then the longitudinal images were captured in videos at 1 Hz, which was the temporal resolution of the current shear wave elastography version and the spatial resolution was 1×1 mm^2. The transducer was slightly moved to acquire a good image. When there were several disconnected muscle fibers through the whole image on the B-mode, the image was considered as elastography with satisfactory quality (Blazevich et al. 2006). A 5-second video of elastography was captured for passive stiffness and a 10-second video for active stiffness. During each measurement, the magnitude of square color box was changed to appropriate size as big as possible according to the muscle thickness. Further analysis achieved the shear modulus from each video as the value of muscle stiffness.

4.3.4 Maximal isometric voluntary contraction (MIVC) test

The participants were positioned supine with hips at 0° flexion and neutral rotation, the pelvis, trunk, and thigh stabilized with straps. The rotation center of the Cybex was aligned with the knee joint rotation center (lateral femoris condyle). To assess the knee extensor strength, the knee joint of participants was set at 30° knee flexion. Prior to measurement, the participants were instructed to perform two submaximal contractions to warm up and become familiar with the test procedure. Then the participant conducted three 5-second maximal contractions with 2-minute intervals between each contraction. The maximum peak torque of the three contractions was recorded as the MIVC. Then the MIVC at 60° and 90° knee flexion was assessed by the same method.

4.3.5 Test protocol

The assessment began with passive muscle stiffness measured after the participant rested for 10 minutes. For each stretch position, the knee joint was kept at the position for 30 seconds before the muscle stiffness evaluation began so that the muscle could get used to the new position. All the participants were instructed to relax their muscles during all the image capture process. Five successive images were captured for further management in video format. Then the participants experienced MIVC estimation.

After that, the active muscle stiffness measurement was followed. The test order of muscle was randomized both in passive and active muscle stiffness measurement. The active muscle stiffness was measured at 30°, 60°, and 90° knee flexion in sequence. Specifically, while the participants extended the knee joint isometrically at loading of 30% MIVC at 30° knee flexion first, the active muscle stiffness of one muscle was measured and three contractions were performed at each angle. Between contractions, a 2-minute interval was provided for rest.

4.3.6 Data analysis

The video format of shear wave elastography were exported and then sorted as images in sequence. Image processing was carried out by a custom Matlab script (MathWorks, Natick, MA). All images were cautiously reviewed to evaluate the quality. If any artefact was detected in any image, the area of region of interest (ROI) was diminished to exclude the artefact from all images within the videos. By this method, a series of coloured two-dimensional images of shear moduli were further processed and then the values of shear modulus in each image were read out. The average of shear moduli in each video was calculated as the muscle stiffness for advanced statistic.

Statistical analysis was managed using 21.0 SPSS software package (New York, USA). Statistical significance level of p value was set to be less than 0.05. Independent sample t-test was employed to compare the difference of basic demographic information, MIVC at each angle between males and females. A three-way repeated measure ANOVA (within subject factors: muscle and knee angle; between subject factor: gender (males and females) was used to compare the passive shear modulus of the three muscles. For active stiffness, two-way mixed measures ANOVA (within subject factors:

muscle; between subject factor: gender (males and females) was used to compare the active shear modulus between genders with 30% MIVC as covariable at each tested angle separately. *Post-hoc* analyses were performed when appropriated using the Bonferroni method.

4.4 Results

The MIVC of males was significantly greater than females at all three angles (Table 4.1). For passive shear modulus, three-way repeated ANOVA analysis revealed a significant main effect of muscle ($p < 0.001$), angle ($p < 0.001$), and gender ($p = 0.010$). There was significant muscle × angle × gender interaction ($p < 0.001$) on passive shear modulus. Follow-up post-hoc test found that the passive shear modulus of RF muscle in males was significantly greater at 60º ($p = 0.019$), and 90º ($p = 0.017$) knee angles than females by 35% and 42%, respectively (Figure 4.2). However, no significant gender differences were detected on the passive shear modulus of VM and VL muscle ($p > 0.05$).

For active shear modulus, two-way repeated ANOVA analysis revealed significant main effect of gender at 60º ($p = 0.024$) and 90º ($p < 0.001$) knee flexion. No significant gender effect was detected at 30º knee flexion ($p = 0.150$). No interaction effect was detected between muscle × gender or muscle × MIVC (all $p > 0.05$). Specifically, greater active stiffness for 21% and 44% for VM were detected in the females at 60º and 90º knee flexion, respectively. The corresponding values were 8% and 16% for RF and 27% and 11% for VL at 60º and 90º knee flexion, respectively.

Table 4. 1 The MIVC of keen extensors between males and females at three tested knee angles (N·m).

Variables	Males	Females	p values
30º	69.2 ± 23.7	46.2 ± 9.5	0.001
60º	112.9 ± 39.0	82.4 ± 18.5	0.007
90º	125.4 ± 49.5	95.2 ± 22.5	0.028

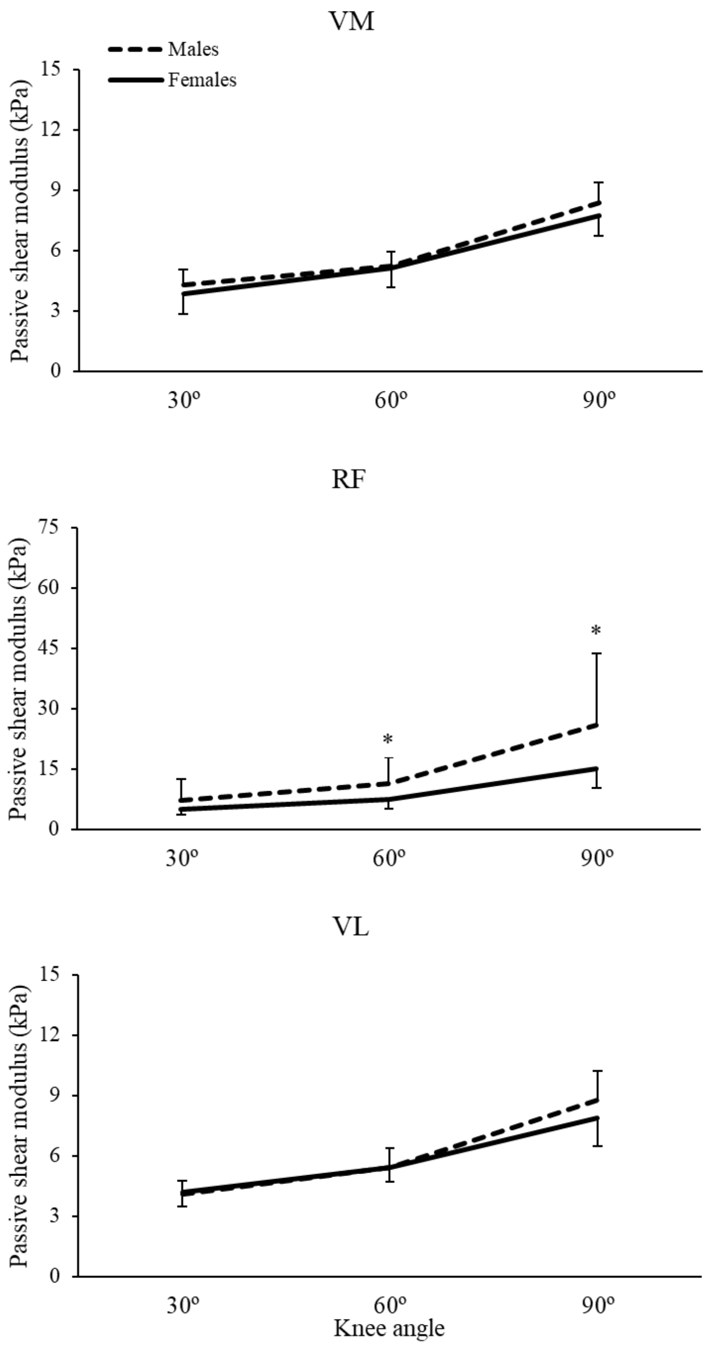

Figure 4. 1 The comparison of passive shear modulus of VM, RF, and VL muscles between males and females. * $p < 0.05$. VM: vastus medialis oblique; RF: rectus femoris; VL: vastus lateralis.

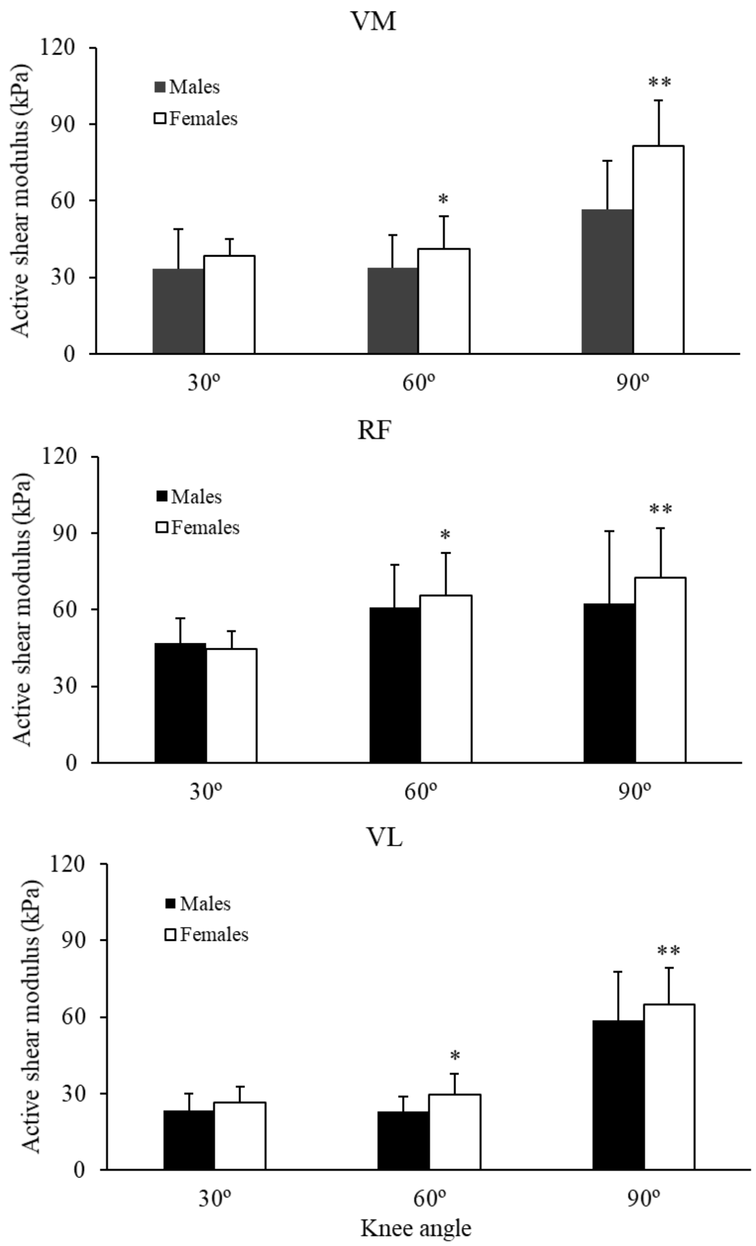

Figure 4. 2 The comparison of active shear modulus of VM, RF, and VL muscles between males and females during isometric knee extension at 30% MIVC with MIVC as covariable at each tested angle. * $p < 0.05$, ** $p < 0.001$. VM: vastus medialis oblique; RF: rectus femoris; VL: vastus lateralis.

4.5 Discussion

The present study revealed that the passive stiffness of RF muscle in males was greater than females when the knee was passively flexed over 60º. Higher active stiffness was observed in the females when the knee was positioned beyond 60º knee flexion than males.

In present study, the quadriceps femoris muscle displayed muscle-specific variation across genders with RF displayed higher passive stiffness in males than females but not VL and VM muscles. Previous studies have reported similar results. Greater stiffness in males than females has been reported by using free oscillation technique for RF (Agyapong-Badu et al. 2016). For VM and VL, healthy young males and females did not show any difference in passive stiffness when measured by shear wave elastography (Botanlioglu et al. 2013). A previous investigation that compared the stiffness difference of isolated muscle across gender might provide some explanation (Martin-San Agustin et al. 2018). The authors measured the maximum radial deformation in VM, RF, and VL, which was an indirect indicator for stiffness of muscle bellies by tensiomyography and found that only RF muscle showed less deformation in males than females. That finding suggested the RF muscle belly from males was stiffer than females. Such findings suggested that gender effect on passive muscle stiffness is muscle-head specific.

There are possible mechanisms in which males presented greater passive stiffness. It has been reported from biopsy studies that the quadriceps femoris muscle of males contains significantly less type I fibers both in percentage and area than females (Roepstorff et al. 2006; Simoneau and Bouchard 1989; Simoneau et al. 1985; Staron et al. 2000); and the stiffness of type I fibers was much higher than type II fibers in animal studies (Iriuchishima et al. 2012; Ramsey et al. 2010). Hence, gender differences in muscle fibre type might not be the main mechanism for findings in present study. In this connection, McHugh et al. (1999) suggested that passive muscle stiffness mainly reflects the extensibility of tendon and aponeurosis paralleled to muscle. Given that greater patellar tendon stiffness has been found in males compared to females (Kubo et al. 2003; Onambele et al. 2007; Tas et al. 2017), the stiffness of patellar tendon may be a factor that contributes to the gender difference of passive stiffness of RF muscle. Anatomically, the tendon from RF existed mostly at the superficial layer of quadriceps tendon and continue as patellar tendon, while the fiber from VM and VL tendons lied in the middle layer (Iriuchishima et al. 2012; Zeiss et al. 1992). The different depth arrangement of tendon from the three muscles might be the cause of divergence of passive stiffness.

The joint angle has been proved to be one of the important factors that influenced passive muscle stiffness by altering muscle length (Hug et al. 2013; Koo et al. 2014; Xu et al. 2018). In the present study, the result indicated that the joint angle contributed to the gender effect on passive stiffness of RF with the difference becoming significant over 60º knee flexion at which muscle was stretched. Consistent effect could also be observed in upper limbs. Eby et al. (2015) have found that the passive stiffness of biceps branchia muscles was affected by gender when the elbow was in an extended position but not in a flexion position. That means at a stretched length, the passive muscle stiffness of biceps branchia muscles were affected by gender. However, their results revealed a tendency of higher stiffness for females than for males, which is reversed with results in this study. The reason for this conflict outcome might be muscle dependent of gender effect on passive stiffness, because for the same participants greater passive stiffness in males than females could only be detected in RF but not biceps branchia muscle (Agyapong-Badu et al. 2016). Further research is needed to demonstrate the variance.

This study shows significantly greater active stiffness in female adults than male adults when muscles contracted at 30% MIVC at 60º and 90º knee flexion. These results were in conflict with previous studies, which have reported greater active stiffness in males than females in quadriceps femoris muscle and hamstring muscles (Blackburn et al. 2004; Granata et al. 2002a;

Granata et al. 2002b). The active muscle stiffness was calculated according to muscle-related damping of oscillatory motion. Due to the difficulty of isolating muscle stiffness from the whole joint, this calculated active stiffness included several other components including the joint, tendon, and the amplitude of perturbation as well (Granata et al. 2002b). The shear wave elastography was used to measure the muscle heads such that the findings would not be contaminated by gender differences on other structures. In this connection, the contracting tibialis anterior muscle did not show any gender difference in active stiffness from 20% to 60% of MIVC (Souron et al. 2016). In this study, MIVC captured at each tested angle was used as co-variable for controlling any loading effects on muscle stiffness. Findings from the present study indicated gender differences in performing isometric contraction at knee flexion beyond the slack angle (Xu et al. 2018). Also, no significant gender and muscle interaction effect was detected. Such findings could not support any divergence of muscle head effects on active stiffness during isometric contraction at the three tested angles.

The present study had some limitations. First, it should be noted that only one contracting intensity (30% MIVC) was performed. Whether the difference would be apparent with the increase of contracting intensity is unclear. Second, the number of male participants were relatively small. it is necessary to further demonstrate the gender difference in muscle stiffness with a larger population and a wider range of contracting intensity. In addition, it should be kept in mind that findings from this study on the quadriceps femoris could not be generalized to other muscle groups.

4.6 Conclusion

Gender has significant effects on passive and active shear modulus. Divergence on gender effect on passive stiffness and active stiffness during isometric contraction is detected. More specifically, the rectus femoris but not the vastus lateralis and medialis muscles in males is greater than females when the knee is passively flexed to a long length. The female adults are having greater active stiffness in superficial quadriceps femoris muscle heads when isometrically contracting at 30% maximal contraction than male adults. Further study is suggested with a larger sample and at higher contraction intensity.

Chapter 5

Relationship between pre-exercise muscle stiffness and muscle damage induced by eccentric exercise

(Published in European Journal of Sport Science)

5.1 Abstract

Purpose: This study aimed to determine whether the post-exercise increase in passive stiffness is homogenously distributed between the synergist muscles in adults and to determine whether pre-exercise passive muscle stiffness is related to the amount of muscle damage induced by an eccentric exercise.

Methods: Fifty healthy adults were randomly assigned to eccentric exercise group or a control group. The passive shear modulus (an index of stiffness) of rectus femoris (RF), vastus lateralis (VL) and vastus medialis oblique (VM) was measured before, immediately after and at 48 hours (48 H) after eccentric exercise. The maximal isometric voluntary knee extension (MIVC) torque was also measured.

Results: Significant reduction in MIVC torque was observed in the eccentric group both at post and 48 H when compared with pre-exercise (both $p < 0.001$). passive shear modulus of RF increased significantly when assessed at 90° of knee flexion at post and 48 H after the eccentric exercise ($p = 0.004$ and 0.008, respectively). Slight but significant decrease in VL passive shear modulus was observed at 30° ($p = 0.01$) and 60° ($p = 0.001$) of knee flexion at post exercise for the eccentric group. No change was observed in VM. The decrease in MIVC at 48 H was negatively correlated with the RF passive shear modulus measured at 90° of knee flexion before the exercise.

Conclusions: Eccentric exercise induced wide range of peak torque reduction and muscle-head specific modulation on passive muscle stiffness. Participants with stiffer RF muscles exhibited lower force generating capacity at 48 H after eccentric exercise.

Key words: Elastography, muscle tension, muscle damage, quadriceps femoris, ultrasound

5.2 Introduction

During eccentric contraction, the muscle is forcibly lengthened leading to damage of its contractile and cytoskeletal constituents (LaStayo et al. 2003). Reduced maximal voluntary contraction (MIVC) torque, delayed onset muscle soreness and increase in passive muscle stiffness are commonly used as non-invasive markers of muscle damage (Proske and Allen 2005). Among them, the decrease in MIVC is considered as one of the best indirect indicators of muscle damage in human (Paulsen et al. 2012). The magnitude of force reduction, however, is highly variable among individuals even when exposed to the same exercise protocol (Clarkson et al. 2005; Nosaka and Newton 2002). The underlying mechanisms for these individual differences in strength loss are unclear. Muscle extensibility has been proposed as one of the factors that affect the force reduction associated with eccentric exercise (Chen et al. 2011a; McHugh et al. 1999).

McHugh et al. (1999) first reported significantly less symptoms of exercise-induced force reduction in participants with more compliant hamstrings than those with stiffer hamstrings. In the same way, Chen et al. (2011a) reported less eccentric exercise-induced force reduction after an 8-week flexibility training program on knee flexors. Further, the maximal range of motion of the hip joint (used as an index of flexibility of knee flexors) was negatively correlated with markers of muscle damage such as decrease in peak torque and plasma creatine kinase activity (Chen et al. 2011a). However, the range of hip flexion is influenced by numerous factors including the stiffness of the muscle-tendon complex as well as the mobility of the hip joint making it difficult to draw a direct relationship between pre-exercise muscle stiffness and the amount of muscle damage. A more direct approach to assess muscle stiffness is needed.

Elastography can be used to estimate the shear modulus of a localized muscle region (Bercoff et al. 2004; Bouillard et al. 2012; Lacourpaille et al. 2014). The shear modulus measured using ultrasound shear wave elastography is strongly linearly related to the Young's modulus (Eby et al. 2013). Using this technique, an increase in passive stiffness was observed after an eccentric exercise (Guilhem et al. 2016; Lacourpaille et al. 2012). The magnitude of this increased stiffness depended on muscle length, i.e., longer the muscle, larger the increase. Using magnetic resonance elastography, Green et al. (2012) observed a muscle-dependent change in passive stiffness after a bout of eccentric exercise. Specially, an increased passive stiffness was observed in the biarticular gastrocnemius muscle but not in the monoarticular soleus muscle. A recent study from Maeo et al. (2018) also reported a significant increase in passive muscle stiffness in the biarticular rectus femoris (RF) muscle but not in the two monoarticular vastus medialis (VM) and lateralis (VL) muscles during single-joint eccentric contraction of the knee extensors.

Considering the fundamental role of the quadriceps muscle group for daily and sports activities, it was interested to investigate whether pre-muscle stiffness of the quadriceps femoris would affect muscle damage induced by eccentric exercise. Specifically, the aim of this study was twofold: 1) to determine whether the post-exercise increase in passive stiffness is homogenously distributed between the heads of the quadriceps and 2) to determine whether pre-exercise passive muscle stiffness is related to the force loss observed after a bout of eccentric knee extensions. The hypotheses would be that: (1) exercise-induced increase in muscle stiffness would be observed in the biarticular rectus femoris (RF) when assessed in its lengthened position but not in the monoarticular heads of the quadriceps muscle (VL and VM) and (2) pre-exercise muscle stiffness assessed in its lengthened position would be negatively correlated with reduction in MIVC torque at 48H.

5.3 Methods

5.3.1 Participants

Fifty-two healthy participants lacking any professional muscle training were recruited in present study. The sample size was estimated through G*Power 3 software (Kiel University, Germany) with effect size equaled to 0.33 (calculated from the pilot study), significance level equaled to 0.05, required power equaled to 0.80. Twenty-four participants in each group was needed. Considering the low dropout of this study, 52 participants were recruited. All the participants did not have any confirmed musculoskeletal disorder or leg injuries. Two participants from the control group did not complete the trial and the data are therefore reported for 50 adults. Participants were randomly assigned to either an eccentric group or a control group by drawing cards. The detailed demographic information is listed in table 5.1.

No significant difference in age, body mass, and height was found between the two groups. However, BMI was significantly higher in the eccentric group than the control group ($p = 0.014$). This study received ethical approval from the Human Subject Ethics Subcommittee of the Polytechnic University of Hong Kong, and all procedures abided by the declaration of Helsinki. All participants provided informed written consent.

Table 5. 1 The demographic information of participants in two groups

	Eccentric group	Control group
N(males)	26 (15)	24 (12)
Age (years old)	23.8 ± 3.3	23.5 ± 3.4
Body mass (kg)	58.6 ± 8.1	56.1 ± 6.6
Height(cm)	166.0 ± 9.2	166.5 ± 8.0
BMI (kg/m^2)	21.4 ± 1.6	20.2 ± 1.2*

* $p < 0.05$

5.3.2 Study design

The study design program is depicted in Figure 5.1. Passive muscle stiffness and peak torque achieved during maximal isometric knee extensions were measured before (pre), immediately after (post) and 48 hours (48 H) after the intervention (eccentric or control according to the group allocation). The order of the measurements was kept the same between the different time points. The intensity of muscle soreness was recorded at 48 H. Reduction in MIVC torque and muscle soreness were used as indirect markers of the amount of eccentric exercise-induced muscle damage. Passive muscle stiffness was assessed using ultrasound shear wave elastography at different knee angles.

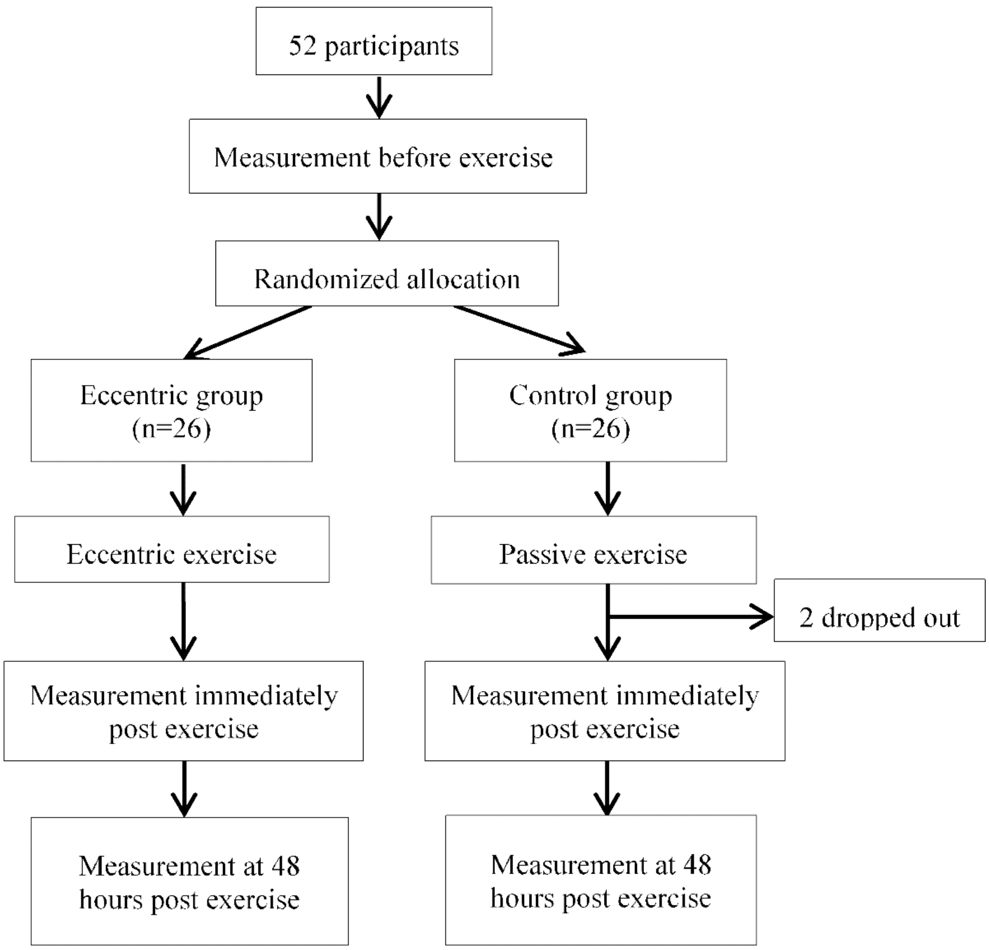

Figure 5. 1 The study design program.

5.3.3 Maximal isometric knee extension force assessment

Participants sat on the chair of an isokinetic dynamometer (Cybex, Medway, Massachusetts, USA) with hip joint was kept at 85° (with supine position = 0°). The knee of the dominant leg was flexed at 60° (0°: knee fully extended). Participants first performed five sub-maximal isometric contractions as a warm-up. These contractions were followed by three maximal isometric contractions for 5 s (2 min rest in-between). The contraction with the highest peak torque was further considered. Peak torque $index_{post}$ and peak torque $index_{48}$ were expressed as percentage of pre-exercise torque.

5.3.4 Muscle stiffness measurement

Muscle shear modulus (an index of stiffness) of the dominant leg was measured by a trained examiner in muscle ultrasound examination who was blinded to the group allocation. An Aixplorer ultrasound scanner coupled with a 4-15 MHz linear transducer array (Aixplorer V4; Supersonic Imagine, Aix-en-Provence, France) was used in the shear wave elastography mode (general preset). Two-dimensional maps of shear modulus, with 1×1 mm spatial resolution, were obtained at 1 sample/s.

During the image acquisition, the participants were positioned supine on the bed of the Cybex dynamometer with their hip in neutral flexion and rotation. The axis of the knee rotation was aligned with the rotation center of the dynamometer. The testing muscles were marked as follows: one fifth of the distance from the midpoint of medial patella border to anterior superior iliac spine for VM, half of the distance from anterior superior iliac spine to the midpoint of the superior tip of the patella for RF; one third of the distance from the midpoint of lateral patella border to anterior superior iliac spine for VL (Xu et al. 2018). The transducer was positioned in line with the direction of the muscle fibers for VL and VM; and in line with the direction of the muscle shortening for RF (Bouillard et al. 2012). For VL and VM, optimal image quality was achieved by slightly moving the transducer until several muscle fascicles could be seen without disconnection through the image (Blazevich et al. 2006). The passive muscle stiffness measurements were carried out in 5 s (i.e. 5 measurements at 1 sample/s) for each muscle and each knee angle. Three angles including 30°, 60° and 90° of knee flexion were measured.

Videos of shear modulus maps were transferred in mp4 format and sequenced into png images. Image processing was performed using a custom Matlab script (MathWorks, Natick, MA). First, each image was carefully inspected for artefacts (saturated values) or missing values (unfilled region within the elasticity map). If artefacts or missing values were present in any image, the region of interest (ROI) was reduced in size to exclude the area of artefact and/or missing values. The ROI was defined for each map as the largest muscle area that avoided fascia, bone and hypo-echoic regions. Image processing converted each pixel of the color map into a value of the shear modulus based on the recorded color scale. The shear modulus was averaged over the ROI for each image and the 5 shear modulus values corresponding to the 5 images were then averaged such that one single value was obtained for each measurement.

5.3.5 Muscle soreness assessment

Muscle soreness was assessed subjectively using a 10-cm visual analogue scale (VAS) anchored with 'no pain' at 0 and 'worst pain imaginable' at 10. Participants were asked to rate their maximal self-perceived pain while walking down the stairs at 48 H after the eccentric exercise (Hafez et al., 2013).

5.3.6 Interventions

The eccentric group carried out a bout of eccentric contractions. Specifically, they seated on the dynamometer chair with their hip was set at 85° (with supine position = 0°). The range of knee motion was set between 30° to 110° of knee flexion to minimize knee discomfort (Skurvydas et al., 2011). A total of 75 maximal eccentric contractions were performed at an angular velocity of 0.26 rad/s (Ballantyne and Shields, 2010; Child et al., 1998). Between each contraction the leg was passively repositioned at 30°.

Each eccentric contraction was separated by a 6 s rest interval. The control group underwent passive movement of the knee joint at a velocity of 0.26 rad/s from 30° to 110° for the same time as eccentric group (20 min).

5.3.7 Statistical analysis

Statistical analyses were performed with 21.0 SPSS software package (New York, USA). Normality testing (Kolmogorov-Smirnov) was used to assess normality of the outcomes. Unpaired-t tests were conducted to assess group differences in terms of age, body mass, height, and body mass index. Chi square analysis was conducted for comparing group difference on gender. A mixed design ANOVA with BMI as covariate was used to determine the effect of eccentric exercise on peak torque (between subject factor: group [eccentric, control]; within-subject factor: time [pre, post, 48 H]). Another mixed design ANOVA with BMI as covariate was used to determine the effect of the eccentric exercise on shear modulus of each muscle (between-subject factor: group [eccentric, control]; within subject factors: time [pre, post, 48 H] and knee angle [30°, 60°, 90°]). When significant interactions were detected, a post-hoc analysis was performed using the Bonferroni method. Partial correlation coefficient test was used to test the relationship between pre-exercise muscle shear modulus and peak torque $index_{post}$ or peak torque $index_{48}$ with gender as a factor to control the force difference between males and females. The level of significance was set at $p < 0.05$.

5.4 Results

A significant group × time interaction ($p < 0.001$) on peak torque was observed. Although there was no significant effect of time for the control group, peak torque decreased significantly at post ($p < 0.001$) and 48 H ($p < 0.001$) compared to pre-exercise for the exercise group (Table 5.2).

At 48 H, 24 (92%) participants of the eccentric group developed muscle soreness associated with stair descent with mean pain scored at 2.8 ± 1.9 of VAS. No participant in the control group indicated pain associated with stair descent (Table 5.2).

Table 5. 2 Peak isometric knee extensor torque and muscle soreness before (Pre), after (Post) and 48 hours (48 H) after exercise.

Group		Peak torque			Muscle soreness at 48 H
		Pre	Post	48 H	
Control	(N·m)	144.6 ± 33.9	142.1 ± 33.9	145.3 ± 33.8	0
	%pre	-	98.3 ± 6.1	100.6 ± 3.6	
Exercise	(N·m)	149.7 ± 32.8	124.0 ± 37.1*	134.8 ± 35.6*	2.8 ± 1.9
	%pre	-	83.2 ± 9.7#	90.5 ± 8.9#	

* Significant difference from the pre-exercise value. # Significant difference from the control group. Values are expressed in mean ± standard deviation.

Figure 5.2 describes passive shear modulus of the 3 muscles at pre-, post and 48 H post-exercise. A significant time × angle × group interaction ($F = 8.24$, $p < 0.001$, partial $\eta^2 = 0.152$) on the RF shear modulus was observed. Post hoc analysis indicated that when compared to pre-intervention, the RF shear modulus measured at 90° of knee flexion in the eccentric group significantly increased at post (37.3 ± 57.1%, $p = 0.004$) and 48 H (22.4 ± 35.5%, $p = 0.005$). Figure 5.3 shows a typical elastography images of RF shear modulus measured at 90° of knee flexion before, immediately after and 48 hours after the eccentric exercise. The RF shear modulus did not change when measured at 30° and 60° of knee flexion ($p = 0.44$ and $p = 0.12$, respectively). No change in RF shear modulus was detected in the control group (all p values > 0.05). For the VL muscle, there was a time × group interaction on shear modulus ($F = 7.51$, $p = 0.001$, partial $\eta^2 = 0.138$). Post hoc revealed a slight (< 10%) but significant decrease in VL shear modulus measured at post- compared to pre-intervention for the eccentric group ($p = 0.002$). No significant change of VL shear modulus was detected in the control group (all p values > 0.40). For the VM muscle, neither a significant interaction nor main effects was detected (all p values > 0.05).

When considering the eccentric group, a significant negative correlation was found between the pre-exercise RF shear modulus measured at 90° of knee flexion and the magnitude of torque reduction at 48 hours ($r = -0.41$, $p = 0.041$) (Figure 5.4). It indicates that the lower pre-exercise RF shear modulus, the less reduction in peak torque induced by eccentric exercise. No correlation was found when considering the other knee angles and times for RF or the other muscles (VM and VL; all $|r| < 0.40$ and all p values > 0.08). No significant correlation was found between shear modulus and muscle soreness (all p values > 0.09).

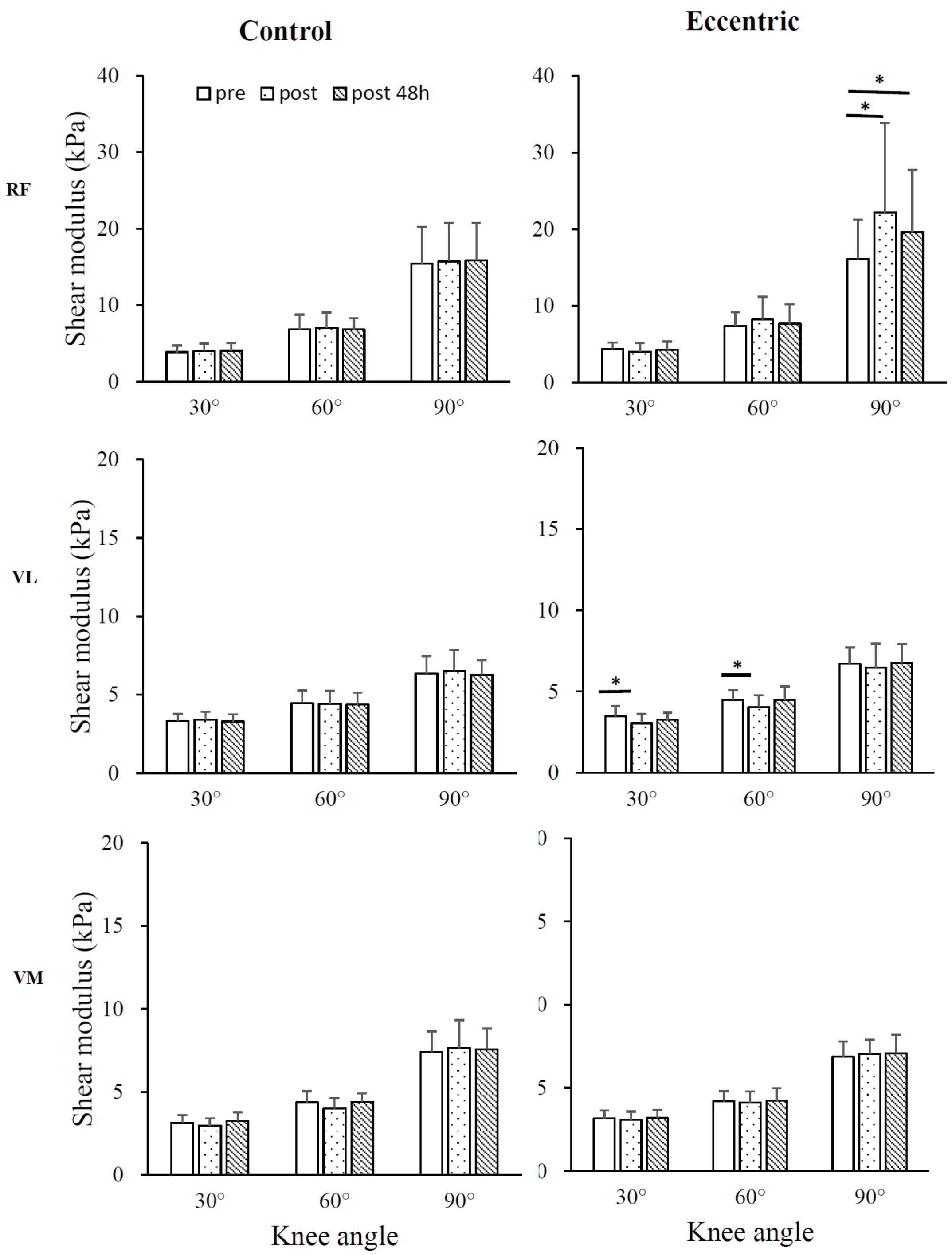

Figure 5. 2 Muscle shear moduli measured at pre, post and at 48 H post- intervention. ∗ indicates significant difference compared with pre exercise. Note: RF: rectus femoris; VL: vastus lateralis; VM: Vastus medialis oblique. ∗ $p \leq 0.01$.

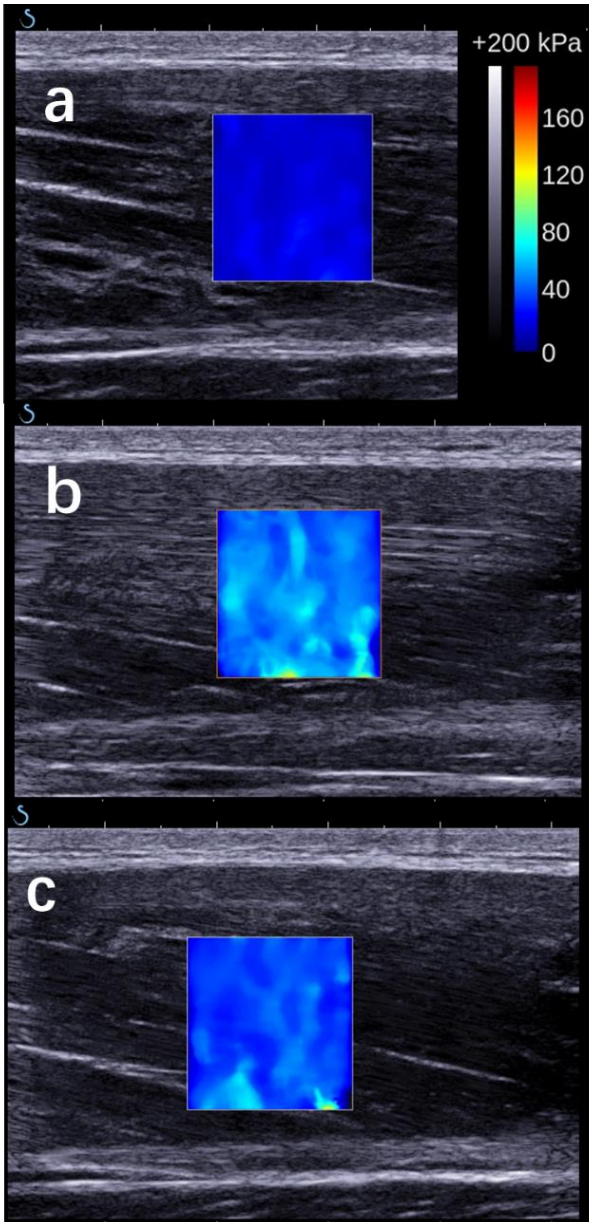

Figure 5. 3 Typical elastography images of RF shear modulus measured at 90° of knee flexion before (a), immediately after (b), and 48H after (c) eccentric exercise.

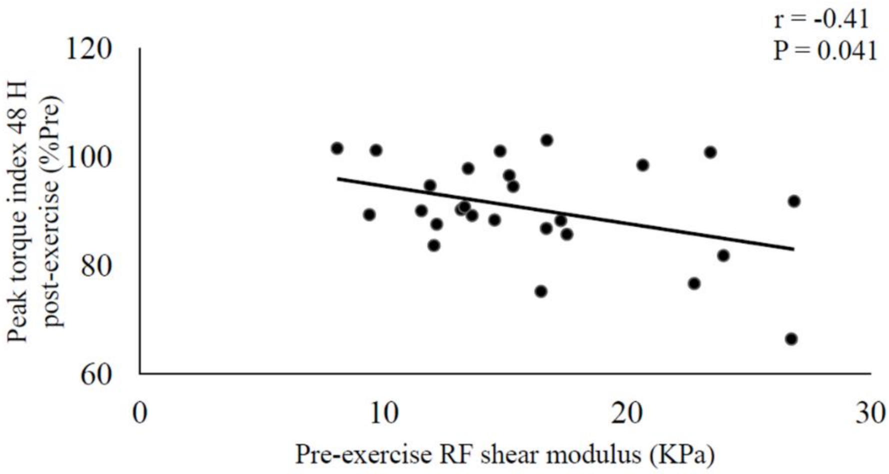

Figure 5. 4 Plotting of peak torque index and passive shear modulus of RF at 90° of knee flexion in participants receiving eccentric exercise. Note: RF, rectus femoris.

5.5 Discussion

Significant reduction in peak torque of knee extensors immediately after and at 48 H after exercise was observed in the eccentric group. The RF shear modulus measured at 90° of knee flexion increased immediately after and 48 H after the bout of eccentric exercise. The effect of eccentric exercise on stiffness was muscle-specific. In addition, the lower pre-exercise RF shear modulus, the less reduction in peak torque induced by eccentric exercise.

In the present study, a decrease in peak torque of about 17% and 10% at post- and 48 H after an eccentric exercise was observed, respectively. This is in line with previous studies that report a decrease in force of about 12% at 48 H after a knee eccentric exercise (Maeo et al. 2018). In accordance with previous work (Clarkson et al. 2005; Nosaka and Newton 2002), a large inter-individual variability in the reduction of peak torque (range: -3% to -33% at 48 H) was observed despite that the participants performed the same eccentric exercise. This variability might be explained by different muscle mechanical properties. For example, the decrease in baseline isometric knee extension torque observed from 70° of knee flexion to 110° has been shown to be correlated with the strength loss observed after a damaging eccentric exercise (McHugh and Pasiakos 2004). This is in line with the sarcomere strain theory of muscle damage in that muscles with a steeper descending limb on the length tension curve would experience greater sarcomere strain for a given exercise and would subsequently exhibit more myofibrillar disruption. In a similar way, the flexibility of the muscle-tendon unit has been suggested as an important factor that affect the susceptibility to exercise-induced muscle damage (Chen et al. 2011a; McHugh et al. 1999). An 8-week static stretching program improved flexibility and attenuated the magnitude of muscle damage induced by a bout of eccentric exercise (Chen et al. 2011a). It suggests that there might exist a relationship between tissue extensibility and exercise-induced force reduction.

Post-exercise increase in muscle stiffness has been observed in several muscle group after eccentric exercise, such as elbow flexors (Chleboun et al. 1998; Howell et al. 1993; Lacourpaille et al. 2014), knee extensors (Hody et al. 2013), knee flexors (Matsuo et al. 2015) and plantar flexors (Hoang et al. 2007). Herein, a significant increase in RF shear modulus by about 37% at post and by about 22% at 48 H was observed when measured at 90° of knee flexion. In contrast to what was observed in RF, no increase in stiffness was observed in VM but a slight decrease in stiffness was observed in the VL. This result is in line with a recent magnetic resonance imaging study, which reported muscle damage mainly localized on RF after eccentric knee extensions while VL was the least affected muscle (Maeo et al. 2018). Similar muscle-specific changes were observed in the triceps surae with increased stiffness of gastrocnemius but not of soleus muscle (Green et al. 2012). With similar technology (shear wave elastography), Lacourpaille et al. (2017) reported a larger increase in stiffness for RF, than both VL and VM after an eccentric exercise. Taking together, it was believed that these results suggest that eccentric exercise-induced modulation on muscle stiffness is muscle-head specific. However, it remains to be demonstrated using direct measures of muscle damage.

Several reasons would be associated to the observed localized increase in RF shear modulus. First, because the fast twitch fibres are more susceptible to eccentric exercise induced muscle damage (Douglas et al. 2017), it is possible that the predominance of fast twitch muscle fibers in the RF muscle makes it more prone to damage than the VM and VL muscles (Friden and Lieber 1998; Johnson et al. 1973). Second, the significant increase of muscle tension and high susceptibility to muscle damage during eccentric contractions in biarticular muscle compared with mono-articular muscle make RF more exposed to muscle damage than VM and VL (Cross et al. 2004; Green et al. 2012). Furthermore, since the stretch effect produced by eccentric contraction may be lower in pennate muscle (Guilhem et al. 2016), the more pennate VM and VL at measured site are in less risk of muscle damage by eccentric exercise compared with RF (Ema et al. 2013a). Finally, because muscles with lesser cross-sectional area are more susceptible to eccentric exercise-induced muscle damage (Chen et al. 2011b), RF， which has been found to be the smallest one among the four heads of quadriceps femoris muscle (Arnold et al. 2010), would have higher risk to be damaged than other larger ones.

On the contrary, VL, which appears the largest cross-sectional area in the quadriceps femoris muscle would be least susceptible to eccentric contraction-induced muscle damage (Maeo et al. 2018). The change in VL stiffness was in a different direction compared to that measured for RF, i.e. a slight (about 10%) but significant reduction. It should be kept in mind that post-exercise changes in stiffness are likely the cumulative consequences of various mechanisms with possible opposite effects. For example, increased muscle temperature induces a decrease in stiffness (Sapin-de Brosses et al. 2010) and muscle damage induces an increase in stiffness. It is therefore possible that the effect of muscle damage was much higher than the effect of muscle temperature for RF and that the effect of muscle damage was slightly lower than the effect of muscle temperature for VL.

Another essential finding of the present study is that the peak torque index at 48 H was negatively correlated with the pre-exercise RF shear modulus measured at its lengthened position. This result indicates that participants with higher RF stiffness exhibit larger force-loss at 48 H after an eccentric exercise. It suggests that passively stretch RF before exercise would cause a long-lasting attenuation of peak torque loss (Chen et al. 2011a). This outcome partially agrees with the previous study which demonstrated a positive correlation between the pre-flexibility of knee flexors and muscle force 1 day to 5 day after eccentric exercise (Chen et al. 2011a). As a lower flexible muscle appears to be stiffer, it was speculated that the pre-stiffness of muscle could be one of predictors of decreased muscle force capacity after eccentric exercise. However, no correlation was found between pre-exercise muscle stiffness and immediately post-exercise force reduction in present study. One possible reason could be that immediately after a bout of eccentric contractions, the decrease of muscle force resulted from not only muscle damage, but also muscle fatigue (Faulkner et al. 1993). At 48 H after exercise, no fatigue exists for the recovery form fatigue completed 3 hours after exercise (Faulkner et al. 1993). Another reason may be the different index was used. The flexibility was measured through straight leg raise range of motion which is not only including the mechanical properties of muscle but hip and knee joint, tendon, fascias while direct measurement of muscle stiffness was made in present study. However, it should be noted that the force loss could only be explained partly by pre-exercise RF stiffness due to the moderate relationship ($r = -0.41$).

The present study requires the consideration of the following limitations. First, previous studies reported a length dependency of changes in muscle stiffness following eccentric exercise, i.e., longer the muscle, larger the increase in stiffness (Lacourpaille et al. 2017). It is therefore possible between-muscle and between-participant differences in stiffness changes were explained (at least partly) by different relative muscle length. Specifically, the absence of VL and VM increased stiffness might be explained by the shorter relative length at which they were measured. However, with respect to previous studies that suggested greater RF damage after eccentric knee extensions (Lacourpaille et al. 2017; Maeo et al. 2018), it was believed that the results really reflect greater RF damage. Second, the magnitude of torque loss was relatively small (only 17% immediately after the exercise). If greater muscle damage was induced, the response of VL and VM might have been varied. Future research can be performed with muscle eccentrically contracting at larger intensity.

5.6 Conclusion

This study confirmed that a bout of eccentric exercise induced an increase in RF muscle stiffness when measured at its lengthened position immediately after and at 48 H after exercise. Such change in muscle stiffness was not observed in VM and VL. The force generating capacity of knee extensors at 48 H was negatively correlated with pre-exercise muscle stiffness of RF muscle. Maintaining high force-generating capacity after eccentric contractions is essential for athletes who need to play continuously. Findings from the present study suggest the important role of muscle stiffness in the development of muscle damage. Prophylactic program of stretching should aim for decreasing RF muscle stiffness which may help to keep relative high level of force generating capacity in the quadriceps muscle at 48 H after eccentric exercise although the underlying mechanism remains to be further investigated.

Chapter 6

Summary and conclusions

6.1 Rationale of the study

The powerful function of quadriceps femoris muscle is indispensable for dealing with basic activities of daily living such as walking, stair-climbing, and squatting. From literature review, it was found that structure, function, and injury rate of each head of quadriceps femoris muscle were different than each other. All the dissimilarity might be related to development of pathologic condition, such as knee osteoarthritis, anterior knee pain. Similarly, the mechanical behavior of individual head of quadriceps femoris muscle could also be different among heads. It has been known that muscle stiffness, which is one of muscle mechanical properties, was related to force production capacity (Alfuraih et al. 2019; Botanlioglu et al. 2013) and common injuries (Mendiguchia et al. 2013). The understanding of mechanical behavior of individual head of quadriceps femoris muscle would benefit both clinical and scientific research. A number of studies have investigated the effect on muscle stiffness associated with age, gender, and eccentric exercise, but with inconclusive results. With the development of the shear wave elastographic technology, individual muscle head could be quantified easily, Therefore, the overall objective of the study was to investigate whether modulation on mechanical properties of quadriceps femoris muscle associated with stretching, aging, gender, and exercise would be muscle-head specific. Knowledge generated from this study would underpin targeted health promotion programme associated with exercise and aging.

6.2 Summary of the studies

The thesis was conducted through four studies.

Study 1: This study first experimented and determined the shear modulus-knee angle relationship and the slack angle of individual heads of the quadriceps (VM, RF, and VL muscles) and whether this differed between the muscles. The shear modulus (an index of muscle stiffness) of VM, RF, and VL muscles in 9 male rowers was measured during passive stretching through which shear modulus-knee angle relationship was determined and the slack angles of each muscle were compared. The results showed that the shear modulus of RF was higher than that of VM and VL when the muscles were stretched over 54°. No significant difference was found between the VM and VL.

The findings of this study indicate the slack angle was similar among individual heads of the quadriceps femoris muscle, which was just over 40º knee flexion. Different pattern of passive tension was observed between mono- and bi-articular muscles.

Study 2: The passive and active muscle shear modulus of VM, RF, and VL in 20 young females and 20 senior females was measured with knee joint at 30°, 60°, and 90°. The MIVC of knee extension and muscle performance (6MWT and FTSST) were measured. The results showed that the passive shear modulus of the senior group was significantly greater by 34% and 56% for the RF; and by 13% and 13% for the VL at 60° and 90° of knee flexion, respectively. The RF passive shear modulus could predict 28.3% of the MIVC measured at 60° of knee flexion across the two age groups. The active shear modulus of VM, RF, and VL at 30º knee flexion in senior females was higher than young females by 10%, 20%, and 21%, respectively. At 30% MIVC contraction, the active stiffness of the three muscles differed between different knee angles.

The findings of this study suggest that the age effect on the passive and active muscle stiffness was muscle and angle specific. Specifically, for passive stiffness, RF and VL muscles in senior females was higher than young females at a stretched length. For active stiffness, however, the three muscles exhibited higher active shear modulus in senior females than young females at short muscle length. In addition, the passive muscle stiffness of RF measured at 60° knee flexion could predict the knee extension force.

Study 3: The part compared the gender effect on passive and active stiffness of VM, RF, and VL muscles in 20 young females and 10 young males. The results revealed that passive shear modulus of RF muscle in males was significantly greater at 60º and 90º knee angles than females. No significant difference was found for the passive shear modulus of VM and VL muscle. However, the active shear modulus of the three muscles was higher in females than males at 60º and 90º knee flexion.

The findings of this study suggest the gender effect on the passive muscle stiffness was muscle and angle specific with the passive stiffness of RF muscle in males was greater than females when the muscle was passively stretched to a long length. However, this gender effect on the passive stiffness was not observed in VM and VL muscles. For active stiffness, the three muscles in females presented greater active shear modulus than males at long muscle length.

Study 4: Whether the post-exercise increase in stiffness is homogenously distributed between RF, VL, and VM muscles was investigated in 26 healthy adults and the relationship between passive muscle stiffness and muscle damage induced by a bout of eccentric exercise was explored by measuring the passive shear modulus of RF, VL, and VM before, immediately after, and at 48 hours after eccentric exercise. The results showed the eccentric exercise induced an increase in passive shear modulus of RF at long muscle length and slight decrease in VL at relative short muscle length. No change was observed in VM. The passive shear modulus of RF before exercise was negatively correlated with the decrease in MIVC at 48 hours.

The findings in this study showed eccentric exercise-induced muscle-head specific and length dependent modulation on passive muscle stiffness. Participants with stiffer RF muscles exhibited lower force generating capacity at 48 hours after eccentric exercise.

As a whole, it was found that the passive stiffness of individual heads of quadriceps femoris muscle was modulated by age, gender, and this modulation mainly occurred at long muscle length at which the knee joint was over slack angles. The findings also indicated that the RF is the main muscle head associated with age and gender differences on passive muscle stiffness. In addition, a bout of eccentric exercise could induce increase in passive stiffness of RF at long muscle length but decrease in VL at short length. More important, the passive stiffness of RF muscle could predict muscle force generation. Minimization of muscle stiffness, in particularly in aged individuals and in male subjects, as well as before eccentric exercises, the RF is the targeted muscle head for any conditioning programme.

Our findings also indicate that in senior subjects, gender differences on active muscle stiffness is muscle length specific.

6.3 Clinical Implication of the research findings

There are some clinical implications from the conclusions of this study. First of all, due to the length and muscle-head dependence of passive stiffness, the measurement of passive muscle stiffness should be conducted in each head and at long muscle length (knee flexion over slack angle for quadriceps) to detect any difference between patients and healthy people. Without measuring the RF muscle, the previous study failed to detect any difference between young healthy and patellofemoral pain syndrome females when only VM and VL muscle stiffness was considered at short muscle length (Botanlioglu et al. 2013).

Second, when quadriceps femoris was assessed in pathological conditions, such as muscle injuries, knee osteoarthritis, patellofemoral syndrome, it should be noted that the different mechanical properties of individual heads may be part of the mechanism of dysfunction. As a result of greater passive muscle stiffness in RF muscle than other muscle heads, and the pre-exercise passive muscle stiffness could predict the post-exercise force loss after eccentric contractions, some interventions targeted the RF such as stretching maneuvers should be applied to decrease the passive muscle of RF and then diminish the force loss after eccentric exercise. This would be beneficial for athletes who need to participate in sport events for a short interval.

Therefore, it is necessary to explore whether there is any difference between patients with muscle injuries, knee osteoarthritis and patellofemoral syndrome, and healthy controls in future studies. In addition, some intervention measures—such as stretching and electric stimulation—could diminish the muscle force loss after eccentric exercise is required to be further demonstrated.

6.4 Limitations of the Study

There are several limitations in the present study. First, myoelectrical activity was not recorded to ensure that the muscles remained relaxed during passive stiffness measurement. However, similar to what was done in previous studies (Koo et al. 2014; Lacourpaille et al. 2013), the participant was verbally instructed to stay relaxed before each knee flexion. In addition, an experimenter 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, there recruitment of participants in the research should be careful considered. In study 1, only 9 male rowers were recruited. Although it has been observed gender has no effect on slack angles of muscle heads in triceps surae (Miyamoto, et al. 2018), it should be careful to generate the results to females. Therefore, in study 2, 3 and 4, the muscle stiffness was measured at 30º as lower slack angle and 60º and 90º an over slack angle to avoid the possible minor difference of slack angle between males and females. In addition, due to the training for rowers is primarily focused on the upper limbs and the training for lower leg is not as much as other athletes such as runners or body builders. The slack angles measured from them could be a reference for general population. In study 2 the participants were not older adults as their ages ranged 50 to 70 years old with mean age at 58.4. This age group was chosen because atrophy was almost consistently apparent and occurred in all the skeletal muscles by 50 years old (Lexell et al. 1988). It has been reported that muscle shear modulus increased with age in people more than 60 years old (Eby et al. 2015). If more participants over age 65 were measured, it was speculated that the age effect on increased passive muscle stiffness would be more noticeable.

Third, another limitation that should be noted is the participants' recruitment. Only females were recruited in study 2. However, it was found that the passive stiffness of RF muscle in young males was greater than young females. Therefore, the conclusion should not be arbitrarily extended to males. Besides, the number of male participants was relatively small. A larger population should be recruited to further demonstrate the gender differences in muscle stiffness.

Furthermore, the active muscle stiffness was measured only at one contracting intensity (30% MIVC). Whether the difference between age and gender groups would be the same with the increase of contracting intensity requires further investigation.

6.5 Significance of the Study

First of all, to our knowledge, this study first examined the muscle head-specific and length-specific muscle mechanical properties of individual heads of the quadriceps femoris muscle using ultrasound shear wave elastography. The slack angles of the individual heads were similar at just over 40º knee joint. Each head should be measured at short or long muscle length (below or over slack angle) to detect any difference of stiffness between patients and healthy people.

Second, the age and gender effect on passive stiffness supported previous knowledge that there was greater stiffness in senior adults than young adults and in males than females. Furthermore, the findings from study 2 and 3 provided evidence that the difference occurred at long muscle length (over slack angle) and was not homogeneous between muscle heads within a muscle group. RF is very important for muscle stiffness of quadriceps. Interventions such as stretching manoeuvres or foam rollers should target at RF.

Third, the thesis explored the effect of age and gender on active stiffness of individual heads of the quadriceps femoris muscle. The preliminary findings revealed the young females exhibited greater active stiffness than senior females at short muscle length and young females presented greater active stiffness than young males at long muscle length. The clinical significance of active stiffness is needed to be further investigated in the future.

Finally, the passive muscle stiffness of RF plays an important role in prediction of isometric muscle force and force generation capacity 48 hours after a bout of eccentric exercise. It is very critical for athletes who need to contest continuously to keep large force-generating capacity after competition, which contains a large amount of eccentric contractions. Training programs such as stretching to decrease passive stiffness of RF should be suggested to maintain relative high-level force output and reduce the force decrease at 48 hours after eccentric exercise in knee extension.

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APPENDIX

1. The ethics approval forms

THE HONG KONG
POLYTECHNIC UNIVERSITY
香港理工大學

To	Fu Siu Ngor (Department of Rehabilitation Sciences)		
From	TSANG Wing Hong Hector, Chair, Departmental Research Committee		
Email	rshtsang@	Date	16-Apr-2015

Application for Ethical Review for Teaching/Research Involving Human Subjects

I write to inform you that approval has been given to your application for human subjects ethics review of the following project for a period from 01-Apr-2015 to 30-Sep-2015:

Project Title:	Effects of static and dynamic stretch on muscle stiffness and hop distance in male rowers: a pilot study
Department:	Department of Rehabilitation Sciences
Principal Investigator:	Fu Siu Ngor
Reference Number:	HSEARS20150323001

Please note that you will be held responsible for the ethical approval granted for the project and the ethical conduct of the personnel involved in the project. In the case of the Co-PI, if any, has also obtained ethical approval for the project, the Co-PI will also assume the responsibility in respect of the ethical approval (in relation to the areas of expertise of respective Co-PI in accordance with the stipulations given by the approving authority).

You are responsible for informing the Departmental Research Committee in advance of any changes in the proposal or procedures which may affect the validity of this ethical approval.

You will receive separate email notification should you be required to obtain fresh approval.

TSANG Wing Hong Hector

Chair

Departmental Research Committee

THE HONG KONG
POLYTECHNIC UNIVERSITY
香港理工大學

To	Fu Siu Ngor (Department of Rehabilitation Sciences)
From	TSANG Wing Hong Hector, Chair, Departmental Research Committee
Email	rshtsang@ Date 20-Apr-2015

Application for Ethical Review for Teaching/Research Involving Human Subjects

I write to inform you that approval has been given to your application for human subjects ethics review of the following project for a period from 01-Jan-2016 to 31-Dec-2018:

Project Title:	Exercise effects on muscle mechanics and control strategy of quadriceps
Department:	Department of Rehabilitation Sciences
Principal Investigator:	Fu Siu Ngor
Reference Number:	HSEARS20150416003

Please note that you will be held responsible for the ethical approval granted for the project and the ethical conduct of the personnel involved in the project. In the case of the Co-PI, if any, has also obtained ethical approval for the project, the Co-PI will also assume the responsibility in respect of the ethical approval (in relation to the areas of expertise of respective Co-PI in accordance with the stipulations given by the approving authority).

You are responsible for informing the Departmental Research Committee in advance of any changes in the proposal or procedures which may affect the validity of this ethical approval.

You will receive separate email notification should you be required to obtain fresh approval.

TSANG Wing Hong Hector

Chair

Departmental Research Committee

There were significant task effects for CES and LT activity (CES: $F_{1,17}=13.83$, $p=0.002$; LT: $F_{1,17}=25.00$, $p=0.000$). Muscle activity of CES was significantly lower, while LT was significantly higher in typing than texting in both groups. Activity of UT was showed a trend to be higher in typing compared with texting, but no statistical difference was found. No side effect and interaction effect were found. Regarding subjective discomfort, Case Group showed significantly higher score than Control Group ($F_{1,17}=5.75$, $p=0.033$), but no difference between tasks.

Conclusion(s): This study confirmed the finding from previous research that people with chronic neck-shoulder pain showed altered motor control while using electronic devices such as desktop computers as well as portable handheld devices such as touchscreen smartphones. Further studies should examine the effect of using different types of touchscreen devices with different screen sizes and input methods, and how these may affect musculoskeletal loading when performing different tasks.

Implications: The results suggest that specific assessment and intervention is needed for people with neck-shoulder pain associated with electronic device use. In providing physiotherapy for patients with neck-shoulder pain, a focus on training efficient neck muscle recruitment strategies during electronic device use is an important element of the overall management strategy.

Keywords: Neck pain; Muscle activity; Touchscreen smartphone

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Ethics approval: Human Ethics Committee of the Hong Kong Polytechnic University. Ethics approval has been obtained from 02-Sep-2013 to 31-Aug-2015.

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Research Report Poster Presentation
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Sunday 3 May 2015 12:15
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MECHANIC BEHAVIORS OF THE VASTUS LATERALIS AND MEDIALIS OBLIQUE MUSCLES DURING PASSIVE STRETCHING

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Background: Passive muscle force is the resistance when a muscle is being stretched. This is an important component of muscle function because it is related to the muscle extensibility and contributes to the global joint stiffness.

Purpose: This study aimed to use Supersonic Shearwave Imaging (SSI) technology to assess change in muscle stiffness to passive stretching in the medial (vastus medialis oblique (VMO)) and lateral (vastus lateral muscles (VL)) heads of the quadriceps femoris.

Methods: Fourteen healthy young subjects (7 men) aged 22.3 to 36.8 were recruited. Subjects were sit on an assessment chair, having the hip at 75° flexion, and the knee flexion/extension axis aligns with the axis of rotation of a Cybex dynamometer (Medway, Massachusetts). An Aixplorer ultrasound scanner (Supersonic Imagine, Aix en Provence, France), coupled with a 50 mm long SL-15-4 linear transducer was set to the SSI mode (musculoskeletal preset) to measure shear modulus of the VM and VL muscles. The transducer was aligned to the muscle fiber direction for vastus muscles (VM and VL). Shear wave elastography and B-mode ultrasound images were acquired simultaneously with the knee joint positioned from 0° to 90° knee flexion at every 15°. 3 images were captured for each muscle at each joint position. The mean of the measured shear elastic modulus (sEM) was used for further analysis. All measurements were taken at the subject's dominant leg with the measurement sequence of the muscles being randomized. Repeated measures analysis of variance was used with muscle and gender as independence variable and sEM at different angle as dependent variable. The level of significance was set as $P<0.05$. Visual detection was used to determine the slack angle when the sEM began to increase.

Results: The sEM of the VMO and VL increased from 11.20 ± 1.90 to 22.74 ± 4.16 kPa and 12.48 ± 3.47 to 20.18 ± 3.62 kPa, respectively when being passively stretched with the knee positioned from 0° to 90° ($P<0.000$). The slack angle of the VMO occurred at 60° and the VL at 75°. Such observation was detected in both genders.

Conclusion(s): Change in muscle stiffness to passive stretching is different in the muscle heads of the quadriceps femoris. The medial heads (VM) seems to have an earlier rise in tension than the lateral (VL) head of the quadriceps femoris.

Implications: Muscle behavior to passive stretching is different between the muscle heads of a muscle. Whether such relationship would be altered by disease or pain waits for further investigation.

Keywords: Supersonic shearwave imaging; Quadriceps femoris muscle; Muscle stiffness

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Ethics approval: This study was approved by the Human Subject Ethics Subcommittee of the Hong Kong Polytechnic University.

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Abstract Form

Title:

Age-related increase in muscle stiffness is different in superficial heads of quadriceps muscle

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Background and purpose:

Muscle has been reported to be increasingly stiff with age in animal and human. This study aimed to explore age-related change in muscle stiffness in superficial heads of quadriceps muscle.

Methods:

A total of 40 healthy females with 20 young (mean = 25.4±2.8) and 20 senior (mean= 58.4±5.5) matched with activity level were recruited. Supersonic shear imaging machine was used to measure the shear modulus (an index of tissue stiffness) of the vastus medialis oblique (VMO), rectus femoris (RF), and vastus lateralis (VL) muscles with knee positioned at 30°, 60°, 90° and 105° of flexion (0° as full extension).

Results:

Repeated measures analyses of variance indicated significant main effects of group, muscle and angle, as well as significant interaction between group× muscle×angle (all $p <$ 0.000). *Post hoc* analysis revealed significantly higher shear modulus of the 3 tested muscles in senior than young groups (all p<0.05). However, the differences were observed in the RF and VL with the knee positioned at 60° and beyond. In the VMO, significant group difference was only observed at 105° of knee flexion. The RF muscle had significantly greater increase than the VL and VMO muscles.

Conclusion:

Age-related increase in muscle stiffness is different in the superficial heads of quadriceps muscle in healthy females. The lateral but not the medial heads of the quadriceps muscle in senior females is stiffer with the knee at 60^0 and 90^0 flexion; the bi-articular muscle shows greater increase than the mono-articular muscles in senior than young females. The effects of these dissimilar age-related changes on muscle stiffness on joint kinematic and mechanic properties waits for further studies.

Exploring Relationship between Skeletal Muscle Passive Tension and Walking Ability in Older Female

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Keywords: Shear wave, passive tension, muscle, function, older
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INTRODUCTION

Knee stability and efficiency on level walking are mainly determined by the force produced by the quadriceps muscle group. It was believed that reduction in quadriceps muscle strength is the main factor associated with reduced functional performance observed in older [1]. However, the force a muscle produces also depends on its passive properties. In older adults, increases in passive stiffness of the muscle-tendon unit have been reported [2]. Such modulation is proposed as one of the mechanisms for the preservation of eccentric strength [3]. Therefore, passive component of knee extensor is postulated to be involved in the function of walking.

METHODS

Older females aged 50-65 were recruited from community population.

Passive muscle tension was assessed by an ultrasound shear-wave elastography system (Axiplorer Version 4.2; Supersonic Imagine). A 4-12MHz linear transducer was used with "MSK" preset. The transducer was placed perpendicular to muscle fiber on the muscle belly of three superficial heads of quadriceps femoris. Subjects were in lying with the hip in 10° flexion. A 10-seconds video was captured when knee positioned to 30°, 60° and 90° of knee flexion (full extension=0°). Muscle passive tension was presented as shear modulus. The walking ability of each subject was determined by the distance covered in six minute (6MWT).

Pearson's r test and single linear regression model was used for statistical analysis.

RESULTS AND DISCUSSION

18 subjects participated in the study (Age: 57.24±4.47yo; BMI: 22.41±2.14kg/m2). Shear modulus (KPa) of each muscle heads are listed in Table 1 (Mean±SD).

Table 1. Shear modulus of quadriceps in different knee angles.

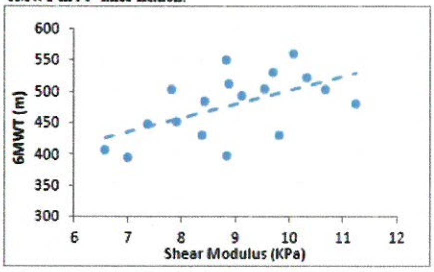
	VL	RF	VMO
30°	4.61±1.16	5.00±1.52	3.74±0.58
60°	6.48±1.61	9.71±2.71	5.16±1.00
90°	9.18±1.79	24.39±5.37	8.93±1.28

VL:Vastus lateralis; RF : Rectus femoris; VMO: Vastus medialis obliquus

Significant correlation can only be found between distance of 6MWT and shear modulus of vastus medialis during 90° knee flexion (p=0.018) but not in the other knee angles. There exists no statistically significant association between 6MWT and shear modulus of either vastus lateralis or rectus femoris.

Single linear model reveals that shear modulus of vastus medialis in 90° is a significant predictor of 6MWT (R^2= 0.305, p=0.018).

Figure 1. Correlation between passive tension of VMO and 6MWT in 90° knee flexion.



In older females, muscle passive tension of VMO in a lengthened position could be a strong indicator to ambulation ability. Vastus medialis is suggested to be primary stabilizer of patellofemoral joint [3]. Increase of passive tension is assumed to a compensatory strategy to maintain knee joint stability.

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Passive Tension of Rectus Femoris is Associated with Lower Limb Function in Young Female

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Keywords: Shear-wave, passive tension, muscle, function

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INTRODUCTION

Sit-to-stand (STS) transition is one of the most common functional activities in daily life, but this movement involves complex sequence of multiple joint. STS requires adequate lower limb muscle, especially knee extensor strength as well as coordination to control large moment transmitting through hip, knee and ankle joint [1-2]. However, the force a muscle produces also depends on its passive viscoelastic properties. The aim of this study is to explore the role of active and passive component of quadriceps in the ability to perform STS.

METHODS

Young females were recruited from local university.

Muscle passive tension was assessed by an ultrasound shear-wave elastography system (Axiplorer Version 4.2; Supersonic Imagine). A 4-12MHz linear transducer was used with "MSK" preset. The transducer was placed perpendicular to muscle fiber on the muscle belly of three superficial heads of quadriceps femoris. Subjects were in lying with hip in 10^0 flexion. A 10-seconds video was captured when knee flexed to 30⁰, 60⁰ and 90⁰. Muscle passive tension was presented as shear modulus. Muscle isometric maximal voluntary contraction (IMVC) was assessed at the corresponding angles. Lower limb ability is determined by the time required in performing five times sit-to-stand (5STST).

Pearson's r test and linear regression model was used for statistical analysis.

RESULTS AND DISCUSSION

20 subjects participated in the study (Age: 25.39±2.83yo; BMI: 20.87±1.70kg/m^2).

There is significant positive correlation between time of 5STST and shear modulus of rectus femoris in 30⁰ flexion (p=0.025) and IMVC in all 3 positions (p=0.47-0.53). No significant result can be found in vastus lateralis or vastus medialis. Multiple linear regression shows that shear modulus of RF and IMVC in 30⁰ are significant factor contributing to performance of 5STST (R^2=0.413, p=0.004).

Figure 1. Correlation between passive tension of RF and 5STST in 30⁰ knee flexion

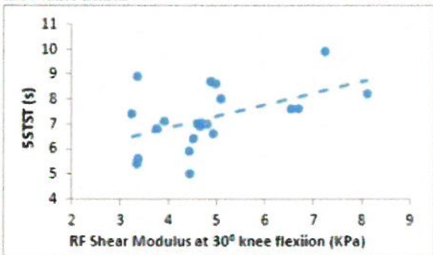
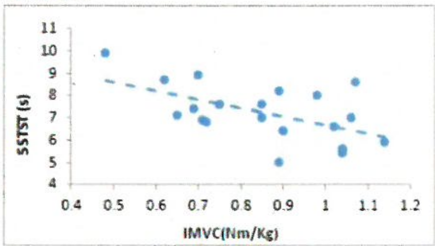


Figure2. Correlation between IMVC of RF and 5STST in 30⁰ knee flexion



Finding from this study indicated that young female with higher passive rectus femoris tension and lower isometric quadriceps muscle strength requires more time in performing sit-to-stand. Rectus femoris crosses both hip and knee joint having higher passive tension might reduce energy conservation during the descending phase. The passive component of rectus femoris and strength of quadriceps muscle strength are essential components for sit-to-stand performance.

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