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**THE ELASTIC PROPERTIES OF PATELLAR
TENDON IN JUMPING ATHLETES WITH AND
WITHOUT PATELLAR TENDINOPATHY**

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Ph.D

The Hong Kong Polytechnic University

2015

THE HONG KONG POLYTECHNIC UNIVERSITY

Department of Rehabilitation Sciences

**THE ELASTIC PROPERTIES OF PATELLAR
TENDON IN JUMPING ATHLETES WITH AND
WITHOUT PATELLAR TENDINOPATHY**

ZHI JIE ZHANG

A Thesis Submitted in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy

December 2014

CERTIFICATE OF ORIGINALITY

The idea of the present study originated from Dr SN Fu. The design of the experiment, the protocol and data interpretation resulted from discussion and multiple trials between the author and Dr SN Fu.

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, that it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

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ABSTRACT

A tendon acts like a spring in absorbing and storing elastic energy for energy efficient movements. In this thesis, the elastic properties of the patellar tendon are assessed on relation to performance enhancement and injury prevention. Because jumping athletes experience a high landing impact, there is a strong rationale for examining the effects of sports participation on the elasticity of the patellar tendon among volleyball and basketball players. If weakness in hip muscles is found in patients with anterior knee pain, a similar observation may also occur in patients with patellar tendinopathy. In addition, the existing findings on the modulation on tendon stiffness in patients with tendinopathy are inconsistent. Most individuals with tendinopathy experience pathological changes and pain at the proximal region of the patellar tendon. Therefore, it is worth re-examining possible regional change in tendon elasticity and its correlation with pain and function. To further establish the relationships between patellar tendon elasticity, pain, and dysfunction, an interventional study is needed to assess whether the changes in the patellar tendon elastic properties are associated with the modulation of pain.

This thesis includes cross-sectional and prospective studies. Supersonic shearwave imaging (SSI) technology was hypothesized to provide reliable measurements on regional tendon elasticity. Using this technology, the elastic properties of patellar tendon in jumping athletes such as volleyball and basketball players was assessed to ascertain whether the elastic properties of patellar tendons were modulated when compared with age- and gender-matched sedentary subjects. The strength of the hip

abductors and external rotators was likely to be weaker in volleyball and basketball players with patellar tendinopathy when compared with healthy players. The muscle strength might also be associated with patellar tendon elasticity. Moreover, the reduced in tendon elasticity in players with unilateral tendinopathy might be associated with the magnitude of pain. Finally, after a session of extracorporeal shockwave therapy, the changes in the patellar tendon elastic properties could relate to the modulation of pain in the treatment group.

Experiment 1: The elastic properties of the eight fresh patellar tendons of pigs were measured by SSI technology and compared with the tangent traction modulus calculated from a material testing system (MTS). Spearman correlation coefficient between the shear elastic modulus from the SSI and tangent traction modulus from the MTS was 0.82 to 1.00 ($p < 0.05$) on the eight tendons. The intra- and inter-operator reliabilities of test-retest measurements were conducted on 11 healthy subjects were 0.98 and 0.97, respectively.

Experiment 2 to 5: Male volleyball and basketball players aged between 18 and 35 were recruited from local university and community teams. All athletes practiced their sports for at least two days per week. Sedentary subjects of similar age and gender, but who did not participated in any regular sporting activity, were recruited from local university community. Athletes with patellar tendinopathy were recruited from the same sport teams. All of the assessments and interventions were conducted at the Hong Kong Polytechnic University.

Experiment 2: The tendon shear elastic modulus of the subjects was measured at the proximal patellar tendon to test tendon elasticity and to compare between sedentary and jumping athletes. Experiment 3: The muscle strength of the hip abductors and external rotators of athletes with and without patellar tendinopathy was measured using a hand-held dynamometer. Experiment 4: The magnitude of pressure pain and activity-related pain were quantified by a hand held algometer and the Victorian Institute of Sport Assessment-patella (VISA-P) questionnaire in players with unilateral patellar tendinopathy. Experiment 5: Tendon shear elastic modulus of the proximal patellar tendon, pressure pain, single-legged declined-squat tests (SLDST) were conducted before and after a session of extracorporeal shock wave therapy (ESWT).

The results from these studies demonstrated that elastic properties of the patellar tendon could be adapted with sports participation. Male individuals with long-term participation in volleyball and basketball had better tendon compliance than sedentary subjects, in that they showed a lower tendon elastic modulus at the proximal patellar tendon. The volleyball players showed better tendon compliance compared with the basketball players (by 24.9%). Age was the only anthropometric factor found to be related with the patellar tendon elasticity of the volleyball players ($r=0.53$; $p=0.003$).

The results further showed that the athletes with patellar tendinopathy had lower normalized isometric muscle strength in the hip abductors and external rotators when compared with the healthy controls. The magnitude of reduction was 18.2% and 11.2% in the hip abductors and external rotators, respectively. In addition, significant increases in the elastic shear modulus of the patellar tendon (by 48.0%) and vastus lateralis muscle (by 26.5%) were detected in players with patellar tendinopathy when compared with

healthy controls. Moreover, a negative correlation was established between the shear elastic modulus of patellar tendon and normalized hip strength, in that weaker hip strength was associated with a stiffer tendon.

In athletes with unilateral patellar tendinopathy, significant correlations were found between the tendon shear elastic modulus ratio (shear elastic modulus of painful over non-painful tendons) and the intensity of pressure pain, VISA-P scores, and the sub-scores of the VISA-P scores for going down stairs, lunges, single leg hopping, and squatting. After a session of ESWT on the patellar tendon, a significant reduction in the tendon shear elastic modulus was observed. More importantly, the reduction in the tendon shear elastic modulus was related to the reduction in squatting pain and the composite change on knee range and squatting pain during the SLDST in the treatment group ($r=0.52$ and 0.59 , respectively). This relationship was not observed in the sham group.

Five main conclusions could be drawn from the study findings: 1) SSI was a reliable technique for measuring regional tendon elastic properties. 2) Jumping athletes had better compliance at the patellar tendon compared with the sedentary subjects. The volleyball players also showed better tendon compliance when compared with the basketball players, which might be related to the different physical activities and demands of the two jumping sports. 3) Decreased in muscle strength of the hip abductors and external rotators was observed in athletes with patellar tendinopathy when compared with healthy controls, and the weakness in these muscles was associated with elasticity in the patellar tendon. 4) Tendon compliance was reduced at the painful site in athletes with patellar tendinopathy and the reduced in tendon compliance was associated with the

magnitude of pain. 5) A session of ESWT induced a reduction in tendon stiffness that was also associated with the reduction in the magnitude of pain. Taking together, with previous findings, these findings indicated that tendon compliance could be modulated. The findings further demonstrated that the reduction in patellar tendon compliance was associated with weakness in the hip muscle and the magnitude of pain. A session of ESWT also induced short-term improvements in tendon compliance and pain. These findings strongly suggest that the strengthening programs for volleyball and basketball players should include exercise for the hip abductors and external rotators muscles. Finally, intervention for improving in tendon compliance, such as ESWT, can be used to individuals with patellar tendinopathy.

RESEARCH OUTPUT ARISING FROM THIS THESIS

PUBLICATIONS

Zhang ZJ, Fu SN. Shear Elastic Modulus on Patellar Tendon Captured from Supersonic Shear Imaging: Correlation with Tangent Traction Modulus Computed from Material Testing System and Test–Retest Reliability. 2013; PLoS ONE 8: e68216.

Zhang ZJ, Ng GY, Lee WC, Fu SN. Changes in morphological and elastic properties of patellar tendon in athletes with unilateral patellar tendinopathy and their relationships with pain and functional disability. PLoS One. 2014;10;9(10):e108337.

Kot BCW, **Zhang ZJ**, Lee AWC, Leung VYF, Fu SN. Elastic Modulus of Muscle and Tendon with Shear Wave Ultrasound Elastography: Variations with Different Technical Settings. PLoS ONE 2012;7: e44348.

CONFERENCE PRESENTATIONS

Zhang ZJ, Kot BCW, Lee AWC, Fu SN. Poster Presentation: ShearWave Ultrasound Elastography of Thigh Muscles: Intra- and Inter-Rater Reliability. 2011 World Federation for Ultrasound in Medicine and Biology, Vienna, Austria.

Zhang ZJ, Lee AWC, Fu SN. Oral presentation: Changes in elastic properties of patellar tendon in athletes with unilateral patellar tendinopathy and its relationship with functional abilities and pain. 2013 Student Conference in Sports Sciences, Medicine and Rehabilitation, HongKong S.A.R, China.

Zhang ZJ, Ng GY, Lee WC, Fu SN. Poster presentation: Changes in stiffness of patellar tendon in athletes with unilateral patellar tendinopathy and their relationships with pain and functional disability. 2014 International Scientific Tendinopathy Symposium, Oxford, UK.

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LIST OF ABBREVIATIONS

AUC: Area under curve

BMI: Body mass index

CI: Confidence interval

CSA: Cross sectional area

EFD: Energy flux density

ESWT: Extracorporeal shock wave therapy

ICC: Intraclass correlation coefficient

MANOVA: Multivariate analysis of variance

MDD: Minimal detectable difference

mJ: Mega Joule

mm²: Millimeter square

MTS: Material testing system

kPa: Kilopascal

p: Significant level

SEM: Shear elastic modulus

SSI: Supersonic shear imaging

PT: Patellar tendinopathy

RF: Rectus femoris

ROC: Receiver operating characteristic curves

ROI: Region of Interest

SD: Standard deviation

SLDST: Single-legged decline squat test

UANOVA: Univariate analysis of variance

VAS: Visual analogue scale

VISA-P: the Victoria Institute of Sport Assessment-patellar

VL: Vastus lateralis

%: Percentage

=: equal

<: Less than

≤: Less or equal to

>: More than

±: Plus and minus

μ: shear elastic modulus

ρ : Density of soft tissue

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CHAPTER 1

INTRODUCTION

1.1 Introduction

The elastic properties of the patellar tendon are essential for shock absorption on landing from a jump and storing kinetic energy for subsequent jumps. Tendon elasticity can respond to the mechanical forces applied to the tendon and adapt to strength training and sport participation. However, the overloading associated with muscle weakness and inflexibility of the functional kinetic chain may induce excessive strain on the tendon. When the tendon adaption was to fail, the pathogenesis may occur. Strain-induced tendinopathies, such as patellar tendinopathy, are one of the most common injuries in athletes. The prevalence of patellar tendinopathy is highest in sports with jumping movements.

1.2 Tendon structure

The patellar tendon is the central portion of the common tendon of the quadriceps muscles, which continues from the inferior pole of the patella to the tibia tuberosity. Hence, the patellar tendon provides a bone to bone connection. This is difference from the Achillis tendon that connects muscle to bone. The tendon is composed of connective tissue within an extracellular matrix. Collagen fibril is the basic unit of the tendon that composes of 65-80% of collagen fibres (mainly Type I collagen) and approximately 1-2% of elastic fibres (Kannus 2000) (Fig. 1.1).

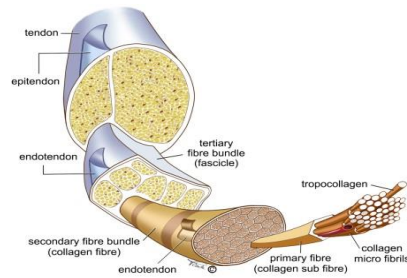


Figure 1.1 The basic structure of the tendon
 (<https://ubcmmedicalart.files.wordpress.com>)

1.3 Function of the patellar tendon

The patellar tendon is an energy-storing tendon that is responsible for transmitting muscle-derived forces that produce joint movement (Alexander 2002; Magnusson et al. 2003), act like a spring in storing elastic energy for energy efficient movements (Arampatzis et al. 2006; Kubo et al. 2010), and function as a “mechanical buffer” to reduce the rate of force transmission to the muscle to prevent muscle injury (Konow et al. 2012; Magnusson et al. 2003). A rigid tendon is better able to transmit the muscle-derived force to the bone, whereas, an elastic tendon can store more energy for energy-efficient movements.

1.3.1 The elastic properties of the patellar tendon and jumping performance

Research has shown that countermovement jump height is related to the elastic properties of the aponeurosis and the tendon. Walshe and Wilson (1997) found a positive relationship between the musculotendinous elasticity and countermovement jump height. The authors suggested that a tendon with more compliance can store and release more kinetic energy for subsequent jumps. In a later study, Kubo et al. (1999) observed that

greater elasticity at the musculotendinous junction of the vastus lateralis was related to higher vertical jump height. Kubo et al. (2007) later reported significant relationship between the elasticity of the Achilles tendon and the height of countermovement and drop jumps. Collectively, these findings provide evidence that aponeurosis and tendon elasticity are essential for jumping performance. An elastic tendon can achieve higher jump heights than a stiff tendon.

1.4 Factors influencing the elastic properties of the patellar tendon

The elastic properties of connective tissues such as the tendon can change with age and the mechanical loading associated with strength training and habitual exercise.

1.4.1 Age-related modulation of tendon compliance

The elastic properties of the tendon have been found to change with aging. For example, Kubo et al. (2007) detected a decrease in the stiffness of the Achilles tendon in older adults when compared with the younger adults. A similar observation was reported by Stenroth et al. (2012), who found the Achilles tendon were 32% less stiff in the older group than the younger group. When measuring the elastic properties of the patellar tendon, Coupe et al. (2009) reported a similar magnitude of difference between old and young subjects, with the older group showing 29% less stiff than the younger group. However, Mian et al. (2007) found no significant difference in elastic properties of muscle-tendon of gastrocnemius medialis between young and old adults. Similarly, Carroll et al. (2008) also reported that there was no significant difference in elastic properties of patellar tendon between young adults and old adults. Thus, the effect of age on the elastic properties of tendon is still inconclusive.

1.4.2 The effect of exercise-related loading on tendon compliance

Physical activities such as strength training and habitual loading can induce mechanical loading on the tendon and alter its elastic properties. Stiffness of the patellar tendon was found to have increased by 65% following a 14-week isometric training program when compared with the control group (Reeves et al. 2003). Eighteen healthy subjects aged 67 to 74 years were recruited in the study. The elastic properties of the patellar tendon were assessed by ultrasound imaging with a dynamometer during muscle contraction. Similarly, three-months' isometric training on the knee extensors with long duration contraction induced a 36.4% increase in the stiffness of the vastus lateralis tendon (Kubo et al. 2001), but no significant change in tendon elasticity was observed with short duration contraction. In contrast, a single bout of hopping exercise did not induce significant changes in the elastic properties of the patellar tendon (Peltonen et al. 2010). The same author also found no significant changes in the elastic properties of Achilles tendon after one hour marathon training (Peltonen et al. 2012). Collectively, these findings suggested that isometric exercise training induced an increase in tendon stiffness when the training is of 12-14 weeks. The duration of contraction may also affect the loading effect on tendon response to loading.

Only one study has investigated the effect of habitual loading on the elastic properties of the patellar tendon. Westh et al. (2008) assessed the elastic properties of the patellar tendon in runners and non-runners. Although the patellar tendon was shown to have greater compliance in the female runners compared with the non-runners (by 14.8%), the difference could not reach a statistically significant level. The results of this study need to be treated with caution because of the small sample size (only 10 subjects

were recruited in each group). To better understand the loading effects of habitual exercise, a study with larger sample size and on sports that involve greater loading on the patellar tendon, such as jumping sports, is needed.

Volleyball and basketball are two common jumping sports that have different physical demands (Kollias et al. 2004; Laffaye et al. 2007). During training and competition, volleyball players were found to take more frequent vertical jumps and land with greater knee flexion (Kollias et al. 2004; Laffaye et al. 2007) than basketball players. Volleyball players were also found to have greater jumping height (Laffaye et al. 2007). These findings indicate that volleyball players may experience more impact loading on the patellar tendon. The elastic properties of the patellar tendon may adapt differently to the sport-specific loadings from volleyball and basketball training. Knowledge of the tendon adaptation to each sport would advance our understanding on the relationship between mechanical loading and tendon adaptation, which is essential for designing sports-targeted, conditioning program for sport enhancement, and injury prevention. In this connection, overloading is the major risk factor for tendinopathy.

1.5 Patellar tendinopathy

Patellar tendinopathy is one of the most common sports injuries (Lian et al. 2005). The condition is diagnosed by a detailed history and physical examination. Clinical diagnosis of patellar tendinopathy includes sport-related pain and focal tenderness in the inferior pole of the patella or proximal insertion of the patellar tendon (Khan et al. 1996; Cook et al. 2000). Jumping, landing and squatting activities, that induce mechanical loading on the tendon are the common pain provoking activities. The diagnosis of patellar

tendinopathy is further confirmed by ultrasound examination. Regions of hypoechoic lesion and vascularization in the proximal part of the patellar tendon are detected in some individuals with patellar tendinopathy (Fig.1.2) (Cook et al. 2004).

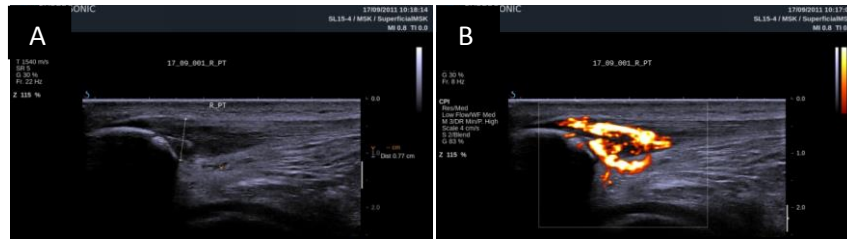


Figure 1.2 Ultrasound imaging of a patellar tendon with tendinopathy. (A) hypoechoic region and (B) increased vascularity.

1.5.1 The prevalence of patellar tendinopathy

The prevalence of patellar tendinopathy was found to be approximately 45% and 32% in elite volleyball players and basketball players, respectively (Lian et al. 2005). Non-elite volleyball players were found to have the highest prevalence among other non-elite athletes (Zwerver et al. 2011). The authors reported an incidence rate of 14.4% in non-elite volleyball players compared to 11.4% in basketball players and 2.5% in soccer players. In addition, male athletes had higher incidence rates than female athletes, at 10.2% and 6.4%, respectively.

1.5.2 The etiology of patellar tendinopathy

The etiology of patellar tendinopathy is still not well understood. Overloading was suggested to be the causative factor in the development of patellar tendinopathy (Lian et al. 1996). However, the risk factors of tendon overloading are considered to be multifactorial. Extrinsic factors, such as training frequency, training duration, training intensity (Neely et al. 1998), and training surface (Lian et al. 2003, Torstensen et al. 1994)

have been investigated and reported. Arthropometry and physical factors were found to be the intrinsic factors related to the overloading of the patellar tendon. The arthropometric factors included increased body weight (Crossley et al. 2007; Malliaras et al. 2007) and waist-to-hip ratio (Gaida et al. 2004; Malliaras et al. 2007). Muscle inflexibility or weakness of the functional kinetic chain can also induce excessive mechanical loading on the patellar tendon. These factors are discussed in the following paragraphs.

1.5.2.1 Physical condition inducing excessive loading on the patellar tendon

Sporting activities, such as the rapid deceleration in landing, place large loads on the lower extremities. The muscle-tendon units of the hip, knee, and ankle joints act to dissipate the kinetic energy on landing (Fredberg et al. 1999). Decker et al. (2003) reported that the knee extensor was the primary shock absorber during landing among male and female subjects, and the hip muscles were the second largest contributor to energy absorption during landing among male subjects (Fig.1.3). About 30% of the energy on landing was absorbed at the hip joint in the male subjects.

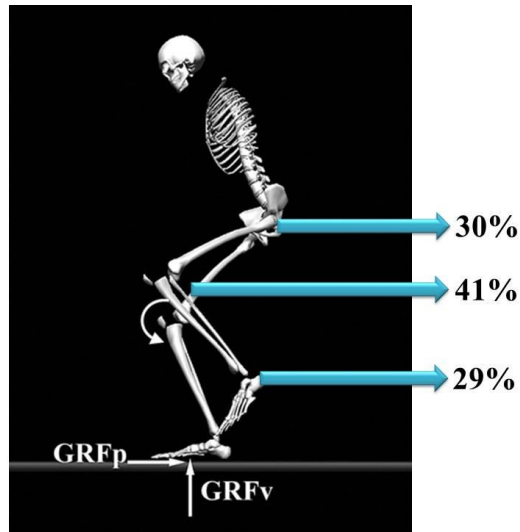


Figure 1.3 Joint energy absorption in the lower extremities during landing

Increased mechanical loading on the patellar tendon can induce deficits in the hip, knee and ankle regions.

1. 5.2.1.1 The hip region

Aside from shock absorption, the hip muscle plays a vital role in landing kinematics (Jacobs et al. 2007). Jacobs et al. (2007) demonstrated that weaker hip abductor strength was associated with increased knee valgus displacement when landing from a jump. Edward et al. (2010) found increased hip adduction and internal rotation and increased knee abduction and internal rotation during horizontal landing in athletes with patellar tendon abnormality compared to those with normal tendons. The authors speculated that the altered kinematics of the hip and knee joints may have increased the loading on the medial side of the proximal patellar tendon during landing. Notably,

patellar tendinopathy was found to commonly occur in the medial part of the patellar tendon among subjects with patellar tendinopathy (Yu et al. 1995). Whether the hip abductor and external rotator muscle are weaker in athletes with patellar tendinopathy has not been investigated.

Nevertheless, impaired hip strength has been identified as a major risk factor in the development of other knee problems such as patellar femoral pain syndrome (Cichanowski et al. 2007; Ireland et al. 2003) and iliotibial band syndrome (Fredericson et al. 2000). Ireland et al. (2003) used a cross-sectional study to explore the association between hip strength and patellofemoral pain and reported a significant difference in the hip strength of subjects with and without patellofemoral pain. Specifically, the hip abduction and external rotator strength have reduced by 26% and 36%, respectively, in female subjects with patellofemoral pain when compared with the healthy group. In a later study, Cichanowski et al. (2007) further confirmed that college level female athletes with patellofemoral pain have weaker strength in the affected hip abduction and external rotation muscle when compared with the non-affected side. In view of the high prevalence of patellar tendinopathy in athletes participating in jumping sports, it is worth exploring whether hip strength is one of the risk factors in the development of patellar tendinopathy in the volleyball and basketball players.

1.5.2.1.2 Reduced thigh muscle flexibility

In a two-year prospective study on 138 collegiate athletes, Witvrouw et al (2001) found the reduced flexibility of the quadriceps and hamstrings contributed to the development of patellar tendinopathy. During the two-year study period, patellar

tendinopathy was found in 19 (13.8%) of the 138 athletes. The patellar tendinopathy group showed reduced flexibility of quadriceps and hamstrings when compared with the control group. A decrease in muscle flexibility was shown to increase the magnitude of loading in patellar tendon during knee joint motion, and contribute to patella tendon overload (Fyfe and Stanish 1992). Van der Worp et al. (2011) proposed that reduced flexibility of the quadriceps muscles is a major risk factor for the development of patellar tendinopathy. Notably, in this study, muscle flexibility was quantified by measuring the range of knee motion using a plastic goniometer. The range of knee motion relies on flexibility of the quadriceps muscles, elasticity of the patellar tendon and mobility of the patella-femoral joint. In addition, the quadriceps are composed of four muscles, this measurement approach cannot identify the source causing the reduction in joint range. To identify where (the muscle, tendon or joint) and which components of the quadriceps muscle is having problem for targeted intervention, direct measurement of the muscle stiffness is needed.

1.5.2.1.3 Ankle dorsiflexion range

Backman and Danielson (2011) showed that a reduction in the ankle dorsiflexion range (less than 36.5°) was associated with 18.5% to 29.4% risk of developing patellar tendinopathy among junior elite basketball players within one year when compared to the players with a dorsiflexion range greater than 36.5°. The authors proposed that the reduced capacity for energy absorption at the ankle joint due to the reduction in the ankle dorsiflexion range may increase the loading on the patellar tendon on landing from jumps.

In regard to the loading mechanics of jump-landing activities, any deficit along the functional kinetic chain may induce excessive loading on the patellar tendon. The patellar tendon may respond by adaptation. However, when adaptation and repair fail, pathogenesis occurs.

1.5.3 Pathogenesis of patellar tendinopathy

In addition to its unclear etiology, the pathogenesis of patellar tendinopathy remains unknown. Histological and morphological changes have been observed in the diseased tendon. Histological studies have demonstrated that patellar tendons with tendinopathy has irregular collagen and poor fiber orientation (Kalebo et al. 1991; Astrom et al. 1995), hypoxic degeneration (Kannus and Jozsa 1991), collagen degeneration (Cetti et al. 2003), and mucoid degeneration (Khan et al. 1996). These findings indicate that patellar tendinopathy is a degenerative rather than an inflammatory disease (Khan et al. 1996; Maffulli et al. 2004). Morphological changes such as an increase in tendon thickness (Cook et al. 2000; Kulig et al. 2013) and the cross-sectional area and physiological modulation in neovascularization (Gisslén and Alfredson 2005; Wang et al. 2002) have also been detected in tendon with tendinopathy.

1.5.4 Changes in the elastic properties of patellar tendons with tendinopathy

Given the above mentioned changes in the histology and morphology of tendons with tendinopathy, the diseased tendon will not have the same matrix and size as normal tendons. These changes may influence the elastic properties of the patellar tendon.

However, only two reported studies have explored the elastic properties of the patellar tendon with tendinopathy. Helland et al. (2013) observed that the pathological

tendons were less stiff than healthy tendons. In their study, 13 male volleyball players with patellar tendinopathy and 15 healthy matched controls were recruited. The elastic properties of the patellar tendon were measured by ultrasonography and a dynamometer. Using the same measuring method, Couppé et al. (2013) did not detect significant difference in the elastic properties of the patellar tendon between seven elite badminton players with patellar tendinopathy and nine healthy players. In both reported studies, the elastic properties of the patellar tendon were assessed using ultrasound imaging with dynamometry. This technique measures the elastic properties of the musculo-tendon-joint complex during ramped maximum voluntary isometric contraction. Clinically, pathological lesions in patellar tendinopathy typically occur at about 5 mm from the apex of the patella (Cook et al. 2000; Fredberg and Stengaard-Pedersen 2008), site-specific evaluation of the pathological region may shed light on the tissue elastic changes associated with tendinopathy.

1.6 Extracorporeal shock wave therapy for patellar tendinopathy

Patellar tendinopathy remains a difficult condition to treat, partly due to its unclear etiology and pathogenesis. The common conservative treatments for patellar tendinopathy are eccentric training and extracorporeal shock wave therapy (ESWT).

1.6.1 Eccentric training for patellar tendinopathy

The eccentric training program was first proposed by Cannell et al. (2001). In the initial study, the subjects were requested to perform for three sets of 15 repetitions of single-legged squats on a 25° declined board, two times a day for 12 weeks. Eight studies have subsequently investigated the effectiveness of eccentric training in treating patellar tendinopathy (Table 1.1). Seven of eight studies were randomized controlled trials. All of the reported studies showed within-group improvement in the eccentric training group. However, only three studies showed significant differences between the exercise and control groups in terms of pain reduction. Five of the reported studies did not detect significant between group differences. The inconsistency in the findings may be due to the different in the treatment protocols (Cannell et al. 2001; Frohm et al. 2007; Young et al. 2005). The efficacy of eccentric training in treating patellar tendinopathy has not been confirmed yet.

Table1. 1 Review of effective of eccentric training in treating patellar tendinopathy

<i>Author</i>	<i>Design</i>	<i>No.</i>	<i>Speed</i>	<i>Knee angle</i>	<i>Follow-up</i>	<i>Result</i>
Cannell et al. 2001	RCT	19	Rapid bend	NT	No	Eccentric group=control group (both improved)
Purdam et al. 2004	Non-RCT	17	Slow	Bend to 90° knee flexion	15 months	Eccentric group >Control group ($p<0.05$)
Stasinopoulos et al. 2004	RCT	30	Slow	Bend to pain tolerant	2 and 4 months	Eccentric group >Control group ($p<0.05$)
Young et al. 2005	RCT	17	Slow	Squat to 60°knee flexion;	12 months	Eccentric group=control group (both improved)
Visnes et al. 2005	RCT	31	Slow	Squat to 90°knee flexion;	6 months	Eccentric group=control group (both improved)
Jonsson et al. 2005	RCT	15	Slow	Squat to 70°knee flexion;	32 months	Eccentric group>control group ($p<0.05$)
Bahr et al. 2006	RCT	35	Slow	Squat to 90°knee flexion;	3,6 and 12 months	Eccentric group=control group (both improved)
Frohm et al. 2006	RCT	35	0.11m/s	Squat to 110°knee flexion;	3months	Eccentric group=control group (both improved)

RCT:randomized control trial

1.6.2 ESWT for patellar tendinopathy

Table 1.2 shows the efficacy of ESWT in treating patellar tendinopathy observed in five reported studies. All of, the studies except from Zwerver et al. (2011) showed that ESWT is effective for treating patellar tendinopathy. However, two of the reported studies were observational studies with no control groups (Peers et al. 2003; Vulpiani et al. 2007). Two of the three randomized controlled trials provided evidence that ESWT was able to reduce pain and improve functional ability among subjects with patellar tendinopathy (Taunton et al. 2003; Wang et al. 2007). Taunton et al. (2003) reported significant improvements in pain, VISA scores and vertical jump height after 3-5 sessions of ESWT with 2000 impulses of 0.17 mJ/mm^2 in ESWT group at three-months' follow-up compared with the sham group (using an energy-absorbing pad). Wang et al. (2007) also found that, after one session of ESWT with 1500 impulses of 0.18 mJ/mm^2 , around 90% of subjects with patellar tendinopathy in the ESWT group were declared to be successfully treated at two- to three-year post-intervention. In the sham group, the success rate was only 50%. The authors also advised the subjects to stop sports for six weeks after intervention. Zwerver et al. (2011) used three sessions of weekly ESWT with 2000 impulses at 0.25 to 0.42 mJ/mm^2 . However, no significant differences in pain and functional ability were detected between the ESWT and sham groups. The authors proposed that the lack of significant findings may have been related to the subjects continuing to participating in sports after the application of the ESWT.

Together, the above studies indicate that when delivered at 0.17 - 0.18 mJ/mm^2 for 1500-2000 impulses for one to five sessions, ESWT seems to be effective in alleviating pain and improving function for patients with patellar tendinopathy.

Table1. 2 Review of extracorporeal shock wave therapy for patellar tendinopathy

Author	Study design	Sample size/ Duration	Protocol	ESWT type	Results on pain reduction	Follow-up
Peers et al.2003	Retrospective cross-sectional analysis	27 13.9m	0.08 mJ/mm ² 3 sessions(1000imp)	Siemens Sonocur Focused	66% improved(<i>p</i> <0.05)	24m
Taunton et al.2003	RCT	20 >3m	0.17 mJ/mm ² 3-5sessions (2000imp)	Siemens Sonocur Focused	70% improved(<i>p</i> <0.05)	3m
Wang et al.2007	RCT	50 16.2m	0.18 mJ/mm ² 1sessions (1500imp)	OssaTron Focused	90% improved(<i>p</i> <0.05)	36m
Vulpiani et al.2007	Non-RCT	73 >3m	0.08-0.44 mJ/mm ² , 3-5sessions (1500-2500imp)	STORZ Focused	79.9%improved(<i>p</i> <0.05)	24m
Zwerver et al.2011	RCT	62 7.5m	Tolerance to 0.25 to0.42 mJ/mm ² 3sessions (2000imp)	Piezowave	ESWT group=Placebo group	5.5m

1.6.2.1 Characteristics of extracorporeal shockwave

A shock wave is a non-linear pressure wave that is oscillated mechanically, and characterized by increasingly high pressure within a short period. Positive pressure impulses from 5 to 100 MPa within 5 ns, and followed by a rapid decrease in pressure of -10MPa (Ogden et al. 2001). The energy flux density (EFD) represents the amount of acoustic energy passing through a 1 mm² area per impulse and is expressed in mJ/mm². Rompe et al. (1998) proposed that ESWT can be classified as low, medium and high energy level when the prescribed EDF is <0.08 mJ/mm², 0.08-0.28 mJ/mm² and 0.29-0.6 mJ/mm², respectively. Pain is normally felt during the application of ESWT. An analgesia injection may be applied when high intensity ESWT is prescribed to alleviate the associated pain (Furia et al. 2008; Schofer et al. 2009). The total energy delivered in a treatment session is the number of pulses multiplied by the energy per pulse (Ogden et al. 2001).

1.6.2.2 Therapeutic mechanisms of extracorporeal shock wave therapy

ESWT has been used to treat chronic tendinopathy since the 1990s. However, the underlying mechanism of ESWT in treating tendinopathies is still not fully understood. Neovascularization, tissue regeneration, nerve destruction and desensitization are the proposed mechanisms (Table 1.3).

Table 1.3 Review of possible therapeutic mechanisms of ESWT for tendinopathy

Author	Year	Specimen	ESWT	Therapeutic mechanism
Neovascularization				
Wang	2002	Achilles tendon	0.18 mJ/mm ² with 1000 impulses	New capillary/vascularized vessels Increased vascularization (4w-8w)
Wang	2003	Achilles tendon	0.12 mJ/mm ² with 500 impulses	eNOS and VEGF /Increased vascularization (4w-12w)
Tissue regeneration				
Chen	2004	Achilles tendon	0.16mJ/mm ² with 200 impulses	Increased TGF-beta1 and IGF-I
Chao	2008	Achilles tendon	0.36mJ/mm ² with 100 impulses	Increased proliferating cell nuclear antigen (PCNA) (at 6 and 24 h) and collagen type 1
Berta	2009	Normal human dermal fibroblast cell	0.22mJ/mm ² with 1000 impulses	Increased mRNA expression/ TGFβ-1/collagen type I(6-9days)
Hsu	2004	Patellar tendon	0.29mJ/mm ² with 1500 impulses	Increased hydroxyproline and pyridinoline concentrations (4w-16w)
Nerve velocity conduction				
Ohtori	2001	Epidermal nerve fibers	0.08mJ/mm ² with 1000 impulses	Reinnervation of the nerve fibers (after 2ws)
Hausdorf	2008	The distal femur	0.9mJ/mm ² with 1500 impulses	A loss of unmyelinated nerve fibers within the femoral nerve (After 6w)
Wu	2008	Sciatic nerves	0.49mJ/mm ² with 2000 impulses	Reduced motor nerve conduction velocity
Neurotransmitters				
Maier	2003	The distal femur	0.9mJ/mm ² with 1500 impulses	Decreased substance P and prostaglandin E(2) (6-24hours)
Hausdorf	2008	The distal femur	High-energy shockwaves	Decreased substance P

Wang et al. (2002) reported that ESWT increased vascularization through the formation of new capillaries and vessels in the medial one-third of the right Achilles tendon-bone junction of dogs. The same research group later reported a significant increase in vascularity in the tendon-bone junction in the Achilles tendon of rabbits (Wang et al. 2003). Changes in vascularisation after ESWT may be attributable to the release of angiogenic growth factors including eNOS and VEGF which would induce vascularization and increase blood supply to promote the healing process in tendon-bone junction (Wang et al. 2003). However, reduction in vascularization was detected when ESWT was applied to patients with supraspinatus tendinopathy (Notarnicola et al. 2011). It is not known whether the modulation in vascularity is different in healthy versus diseased tendons.

Numerous studies have examined the ESWT induced enhancement of tissue regeneration. For example, Chen et al. (2004) observed an increase in transforming growth factor-beta 1 (TGF- β 1) expression and insulin-like growth factor-I (IGF-I) growth factors, which are responsible for up-regulating the extracellular matrix and tenocyte proliferation after the application of ESWT. They also found a significant increase in proliferating cell nuclear antigen (PCNA) in the ESWT group compared with the control group, suggesting that ESWT can raise the mitogenic response to stimulate tenocyte growth and promote the formation of the tendon microstructure. Similarly, Caminoto et al. (2005) also found an increase in TGF- β 1 expression and observed newly formed collagen fibril was observed in ESWT at four weeks of treatment. Using a rabbit model, Hsu et al. (2004) investigated the effects of ESWT on patellar tendinopathy. In the study, ESWT with 1500 impulses at $0.29\text{mJ}/\text{mm}^2$ was applied on rabbits with patellar tendinopathy.

The ultimate tensile load was found to be greater in the ESWT than the in sham group at the 4th and the 6th weeks. In addition, increases in hydroxyproline and pyridinoline concentrations were observed in the ESWT group. Hydroxyproline and pyridinoline concentrations are associated with the formation of collagen and crosslinks (Eyre et al. 1984).

The destruction of nerve fibers and damaged nerve endings have been proposed as the mechanisms for the pain reduction from ESWT (Ohtori et al. 2001; Hausdor et al. 2008; Wu et al. 2008). Ohtori et al. (2001) examined the effects of ESWT on nerve fibers. They applied ESWT with 1000 impulses at 0.08mJ/mm^2 to the plantar skin of rats and observed complete degeneration of the epidermal nerve fibers in the ESWT group. Reinnervation of the nerve fibers occurred two weeks after the ESWT. These findings indicated that pain reduction after the ESWT may attributable to the rapid degeneration of nerve fibers. When high energy ESWT (of 0.9mJ/mm^2) was applied for 1500 impulses on the distal femur, Hausdorf et al. (2008) observed a loss of unmyelinated nerve fibers within the femoral nerve. Similarly, Wu et al. (2008) found that high energy ESWT with 2000 impulses at 0.49mJ/mm^2 damaged the sciatic nerves and reduced motor nerve conduction velocity, which would revert within two weeks. Pain relief using low to medium ESWT may partly be explained by the destruction of the nerve endings.

A hyperstimulation analgesic effect has also been proposed as one of the treatment mechanisms of ESWT (Ogden et al. 2001; Notarnicola and Moretti 2012; Zwerver et al. 2011). This analgesic effect is due to the hyperstimulation of the nociceptors induced by the ESWs. To date, no study has been reported to confirm this hypothesis.

1.7 Pain induced motor and neural consequences

The motor and neural consequences of pain have been investigated, mainly with experimentally induced pain. In animal studies, a decrease in the firing rate of muscle alpha-motoneurons (Kniffki et al. 1981) and a reduction in the sensibility of muscle spindles (Mense and Skeppar et al. 1991) were observed with experimentally induced pain. In human studies, when hypertonic saline was injected in muscles, inhibition of muscle activities in the tibialis anterior muscle (Graven-Nielsen et al. 1997) and the upper trapezius muscle (Madeleine et al. 2006) were detected during static and dynamic contraction. Pain induced by the injection of saline into the infrapatellar fat pad was also found to alter the delay in the muscle onset of the vastus medialis and the reduction in the muscle activities in the vastus lateralis muscles during stepping activities (Hodges et al. 2009). These findings suggest that the alteration of the muscle activities can be induced by pain from non-muscle origins, suggesting that alterations in muscle activation may be due to central rather than peripheral effects. Furthermore, modulation of the corticospinal excitability of the muscles in the contralateral limb was reported in the hand (Kofler et al. 2001) and arm (Hoeger Bement et al. 2009) muscles with experimentally induced pain. If experimentally induced pain does modulate muscle activities, ESWT, a painful intervention, may induce the reduction of the muscle activities in the ipsilateral and contralateral limbs. Such information would shed light on the therapeutic mechanism of ESWT.

1.8 Evaluation of the elastic properties of the patellar tendon

Different types of equipment, from the clinically viable to more complex systems, have been developed, to measure tissue elastic properties. The material testing system is

the most direct and valid method for assessing *in-vitro* tendon elastic properties (Wren et al. 2003; Thambyah et al. (2000). However, this method cannot be applied on human study. Hansen et al. (2006) proposed the use of ultrasonography with a dynamometer to measure the elastic properties of human patellar tendon. With this approach, ultrasound imaging is used to track tendon elongation during muscle contraction, while the muscle contraction force is captured from the dynamometer. Tendon stiffness and the Young's modulus are computed from the change in force per unit area in relation to the change in length. This method has been used to assess the changes in tendon elastic properties associated with ageing (Carroll et al. 2008; Couppé et al. 2009; Kubo et al. 2007), exercise (Couppé et al.2008; Reeves et al. 2003) and tendinopathy (Helland et al. 2013; Kulig et al. 2013). This method requires a long acquisition time (Hansen et al. 2006). In addition, the computed stiffness reflects the stiffness of the muscle-tendon-joint complex.

Direct measurement of the tendon elastic properties has been made possible with the recent development of ultrasound elastography. Here, a compression force is induced by an ultrasound probe and the displacement of the tissues is captured from the ultrasound images. A strain map is then produced to show a strain ratio (Ophir et al. 1991). This method has been used to measure the tendon elastic properties of the Achilles tendon (Drakonaki et al. 2009; De Zordo et al. 2009; Tan et al. 2012), supraspinatus tendon (Muraki et al. 2014) and common wrist extensors (De Zordo et al. 2009). However, this method is operator-dependent, lacks reproducibility and lacks an actual value of tendon elastic properties (Drakonaki et al. 2009; Klauser et al. 2010).

Supersonic shear imaging (SSI) operates on the principle of transient elastography. SSI produces elastography images based on the combination of a radiation force and an

ultrafast ultrasound acquisition imaging system capable of capturing, the propagation of the resulting shear waves in real time (Bercoff et al. 2004) (Fig. 1.4). The elastic modulus (E) is computed from the system using the following equation: $E=3\rho V^2$, where density ρ is assumed to be constant (1000kg/m^3) in human soft tissue and V is the velocity of the shear wave propagation (Bercoff et al. 2004). The elastic modulus can be calculated from the velocity of the propagating wave, when a faster velocity indicates a greater elastic modulus. The unit is kilopascals (kPa).

SSI is a valid and reliable technique for evaluating the elastic properties of skeletal muscle (Eby et al. 2013). Eby et al. (2013) found a significant correlation between Young's modulus from a material testing system and the shear elastic modulus from SSI. Furthermore, Nordez et al. (2010) reported a significant linear regression between shear elastic modulus and EMG activity during sub-maximal contraction of biceps muscles. The test-retest reliability of SSI has been confirmed on most muscles including the quadriceps muscles (Koo et al. 2013; Lacourpaille et al. 2012). SSI is a potential technology for assessing the elastic properties of the patellar tendon.

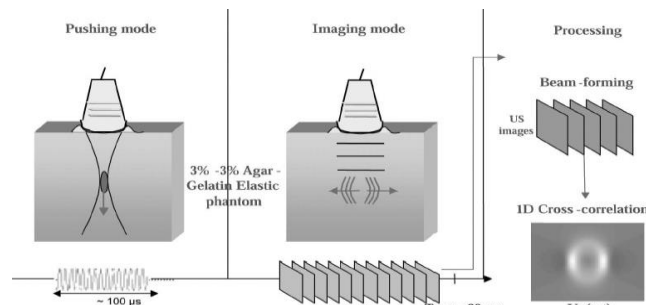


Figure 1.4 The imaging sequence of the shearwave images from the Supersonic Shearwave Imaging System (adopted from Bercoff et al. 2004).

1.9 Rationales and objectives of this study

1.9.1 Rationales of this study

Tendinopathy results in disruption and disorganization of the tendon fibers. Previous studies have reported that the elastic properties of patellar tendon were assessed by the ultrasound imaging with dynamometer, but such approach measures the elastic properties of whole tendon during ramped maximum voluntary isometric contraction. Clinically, pathological lesions in patellar tendinopathy commonly occur in the insertion of patellar tendon. Therefore, site-specific assessment at the pathological region might shed light on the changes on tissue elastic properties related to tendinopathy. SSI can provide quantitative values of tendon elastic properties as a selected area of interest. Therefore, the SSI could evaluate the regional-specific tendon elasticity among subjects with patellar tendinopathy.

The elastic properties of the patellar tendon are essential for shock absorption on landing from a jump and storing kinetic energy for subsequent jumps. Tendon elasticity has been found respond to the mechanical loading applied to the tendon and to adapt to strength training. Participation in sports, particularly jumping sports, can induce mechanical loading on the patellar tendon resulting in adaptation. The lack of significant findings in the previous studies may be related to the small sample sizes and the indirect measurement of the tendon elastic properties. Knowledge of tendon adaptation to sport would advance our understanding of the relationship between mechanical loading and tendon adaptation. Such information is essential for designing sport-targeted injury prevention programs for strain-induced tendinopathy.

Overloading is the one of main causes of patellar tendinopathy. Reduced hip muscle strength and quadriceps flexibility are the proposed intrinsic factors for overloading the patellar tendon. Altered hip kinematic on landing has also been observed in patients with patellar tendinopathy that may induce excessive loading on the patellar tendon. Therefore, weakness of the hip abductors and external rotators muscles is more likely to occur in subjects with patellar tendinopathy than those without.

Changes in tissue histology and morphology have been reported in tendons with tendinopathy: a diseased tendon does not have the same matrix and size as a normal tendon. These changes may influence the elastic properties of the patellar tendon. However, only two studies have been conducted, using small sample size and an indirect measurement method. Whether the tendon elastic properties are altered and how the change is related to the tendon pain await further investigation.

ESWT appears to be a safe and effective intervention for patellar tendinopathy. However, the treatment mechanism is unclear. A hyperstimulation analgesic effect has also been proposed as one of the treatment mechanisms of ESWT. The analgesic effect is due to the hyperstimulation of the nociceptors induced from the ESWs. Indeed, motor and neural consequences have been observed with experimentally-induced pain. Reductions in muscle and tendon elastic properties may occur due to a decrease in the firing rate of muscle alpha-motoneurons and/or a reduction of the sensibility of muscle spindles at the treated limb. Therefore, one session ESWT would modulate the elastic properties of tendon and muscle as well as explore correlation between the changes of elastic properties of tendon and pain intensity among subjects with patellar tendinopathy. The findings would shed light on the therapeutic mechanisms of ESWT.

1.9.2 Aims of this study

The research aims of this study were as follows:

1. to assess whether SSI could be used to provide reliable and quantitative assessments of elastic properties of patellar tendon;
2. to examine adaptation of the patellar tendon elastic properties induced by mechanical loading from habitual jumping using volleyball and basketball players as examples;
3. to compare the strength of hip abductors and external rotators, the muscle elasticity of the vastus lateralis and rectus femoris, and tendon stiffness in athlete with patellar tendinopathy;
4. to detect the modulation of the tendon elastic properties and examine how these changes relate to tendon pain in athletes with unilateral patellar tendinopathy;
5. to explore immediate effects of ESWT on tendon and muscle stiffness and to correlate the changes in tendon elasticity with the associated improvement in pain.

1.9.3 Hypotheses of this study

The above aims were addressed in five related studies based on the following research hypotheses:

- 1) SSI would be a reliable method for directly quantifying the elastic properties of regional-specific patellar tendon.
- 2) The elastic properties of patellar tendon were adapted in athletes participating in jumping sports. The adaptation differs between volleyball and basketball players.

- 3) Weaker hip abductor and external rotator muscles and stiffer vastus lateralis and rectus femoris muscles were detected in male volleyball and basketball players with patellar tendinopathy when compared with age- and activity-matched healthy controls.
- 4) The elastic properties of patellar tendon were different in male subjects with and without unilateral patellar tendinopathy. The changes in the patellar tendon elastic properties were associated the magnitude of tendon pain.
- 5) A single session of ESWT when prescribed at maximum pain tolerable intensity induced changes in tendon and muscle stiffness. The change in elastic properties of the patellar tendons of subjects was related to the change in activity-related pain.

1. 9.4 The structure of the enquiry

This study consists of five inter-related studies

Study 1 (Chapter 2) Elastic modulus on patellar tendon captured from Supersonic Shear Imaging: correlation with tangent traction modulus computed from material testing system and test-retest reliability.

Objective 1: To assess the correlation of the elastic modulus captured from SSI and the tangent traction modulus from a material testing system.

Objective 2: To assess the reliability of the elastic modulus captured by SSI using test-retest measurements on the patellar tendons.

Study 2 (Chapter 3): Elastic properties of the proximal patellar tendon: Effects of age, BMI, leg dominance and sports participation.

Objective 1: To compare the elastic properties of the patellar tendons among sedentary subjects, volleyball and basketball players.

Objective 2: To examine correlations between the elastic properties of the proximal patellar tendon with age, body mass index and training intensity among players.

Study 3 (Chapter 4) Hip strength in male athletes with and without patellar tendinopathy and its association with the elastic properties of proximal patellar tendon.

Objective 1: To compare hip muscle strength (abductor and external rotator), quadriceps muscle stiffness, and patellar tendon elastic properties in athletes with and without patellar tendinopathy

Objective 2: To determine the optimal cutoff points in hip muscle strength, quadriceps muscles, and patellar tendon stiffness for identifying athletes with and without patellar tendinopathy.

Objective 3: To explore possible associations among hip muscle strength, the elastic properties of quadriceps muscles, and the patellar tendon.

Study 4 (Chapter 5) Changes in morphological and elastic properties of proximal patellar tendon in athletes with unilateral patellar tendinopathy and their relationships with pain and functional disability.

Objective 1: To explore changes in the elastic properties of the patellar tendon in athletes with unilateral patellar tendinopathy

Objective 2: To explore associations among changes in tendon shear elastic modulus, pain, and dysfunction in athletes with unilateral patellar tendinopathy

Study 5 (Chapter 6) Immediate effects of one session of extracorporeal shock wave therapy in athletes with chronic patellar tendinopathy

Objective 1: To determine the effects of a single session ESWT on the elastic properties of the quadriceps muscle and proximal patellar tendon; as well as pressure pain in athletes with patellar tendinopathy.

Objective 2: To explore whether change in the elastic properties of the proximal patellar tendon would be associated with changes on the elastic properties of the quadriceps muscles and intensity of pain.

These five inter-related studies are reported in subsequent chapters in terms of the sequence listed under 1.8.4. The main findings of the studies are highlighted and summarized in Chapter 7. The contributions of this study and the generalizability of the findings are also discussed in the chapter 7, along with recommendation for further research.

CHAPTER 2

ELASTIC MODULUS ON PATELLAR TENDON CAPTURED FROM SUPERSONIC SHEAR IMAGING: CORRELATION WITH TANGENT TRACTION MODULUS COMPUTED FROM MATERIAL TESTING SYSTEM AND TEST-RETEST RELIABILITY

2.1 Abstract

Objectives: The objectives of this study were to assess the correlation of the elastic modulus captured from an SSI and the tangent traction modulus from a material testing system (MTS); and to assess the reliability of the elastic modulus captured from the SSI, by using test-retest measurements on the patellar tendons of healthy subjects.

Methods: Eight fresh patellar pig tendons were dissected carefully. The patella and tibia of the dissected patellar tendon were connected to the 2 clamps with their fibers aligned using applied force. The tensile force (F) was applied to the 2 crossheads causing incremental displacement of the 2 clamps (d) in steps of 0.2mm at a test speed of 20mm/min. The force was captured by the load cells and the displacement of the crossheads was measured by an extensometer. Both values were displayed on-line on a computer attached to the MTS. The tangent modulus was computed by a self-written programme based on the formula $E = \Delta \text{tensile stress} / \Delta \text{tensile strain}$. The elastic modulus of pig patellar tendon was assessed by the supersonic shearwave imaging (SSI).

Eleven healthy subjects (8 male, 3 female; age: 26.1 ± 3.2 years, weight: 58.7 ± 12.3 kg, height: 169.2 ± 10.0 cm) were invited to participate in this study. Each participant was examined while lying supine with the knee at 30° of flexion. The elastic modulus of the patellar tendon was measured using the SSI. Two operators (I and II) participated in the inter-operator investigation. The operators took turns to examine each subject's patellar tendon at one-hour intervals; and by Operator II with a 3-hour interval. Spearman's rank correlation tests were used to assess the level of correlation between the elastic modulus of the tendon captured from the SSI system and the tangent traction modulus calculated from the MTS.

Results: Spearman Correlation coefficients for the elastic modulus and tangent traction modulus ranged from 0.82 to 1.00 (all $p < 0.05$) on the 8 tendons. The intra and inter-operator reliabilities were 0.98 (95% CI: 0.93–0.99) and 0.97 (95% CI: 0.93–0.98) respectively.

Conclusions: The present study has indicated that the elastic modulus on the patellar tendon measured from the SSI is related closely to the tangent traction modulus calculated from the MTS. The *in-vivo* measurement has illustrated excellent reliability of this tool. The SSI can be applied to evaluate the elastic properties of a healthy patellar tendon. The diagnostic role of this technique will be investigated by assessing the shear elastic modulus of normal and pathological tendons, as well as to monitor disease progression and the efficacy of intervention on individuals with tendon disorders.

Keywords: Elastic modulus, patellar tendon, supersonic shear imaging, tangent traction modulus, material testing system.

2.2 Introduction

Tendons are involved in every human motion and subjected to high loads. A tendon consists of parallel collagen fibers to resist elongation (Calve et al. 2004) and exhibits viscoelastic properties for force production and absorption (Zajac 1989). Alteration in tendon stiffness may compromise the tendon's capacity to absorb and respond to loads (Arya and Kulig 2010; Maganaris et al. 2002). Quantification of its elastic properties may help improve our understanding of the underlying causes of tendon-related disorders, such as tendinopathy.

The elastic properties of tendons have been determined using animal (Wren et al. 2003) and cadaveric (Thambyah et al. 2000) tendons undergoing ramped stretching imposed by a motor of a material testing system (MTS). It has not yet been established, however, whether findings from isolated excised tendons can be applied to *in-vivo* physiological functions (Maganaris et al. 2002). Ultrasonography is a non-invasive method for measuring the elastic properties of the human tendon *in-vivo* (Maganaris et al. 2002; Hansen et al. 2006). This method has been used to examine changes in tendon stiffness associated with exercise (Reeves et al. 2003) and aging (Carroll et al. 2008). However, complex methodologies and long acquisition time are the drawbacks of this approach (Hansen et al. 2006).

Recently, ultrasound elastography has been applied to investigate the mechanical properties of the Achilles tendon (Drakonaki et al. 2009). Ultrasound elastography (strain imaging) is a real-time imaging tool for the *in vivo* estimation of tissue strain distribution (Itoh et al. 2006; Hall et al. 2003). A compressive force is applied to the tissue surface inducing transverse tissue displacement, which is calculated from the echo signal set

before and after the compression (Ophir et al. 1991). The force can be applied manually (freehand elastography) or mechanically (transient elastography). The absolute value of the elastic properties cannot be provided from freehand ultrasound elastography. Manual compression may alter the mechanical properties of the testing tissues.

Supersonic shear imaging (SSI) operates on a transient elastography principle. It produces elastography images based on the combination of a radiation force and an ultrafast ultrasound acquisition imaging system capable of capturing in real time, the propagation of the resulting shear waves (Bercoff et al. 2004). The elastic modulus can be calculated from the velocity of the propagating wave when a faster velocity indicates a greater elastic modulus. Therefore, the elastic modulus can be calculated by measuring the propagation of shear waves. A light touch on the skin with the ultrasound probe is suggested by the manufacturer and a quantitative elasticity map can be computed from the system within a few milliseconds. The objectives of this study were: (1) to assess the correlation of the elastic modulus captured from an SSI and the tangent traction modulus from an MTS (Experiment I); and (2) to assess the reliability of the elastic modulus captured from the SSI, by using test-retest measurements on the patellar tendons of healthy subjects (Experiment II). We hypothesized that there would be a significant correlation between shear elastic modulus from the SSI and tangent traction modulus from a MTS and the SSI would be a reliable tool to assess the shear elastic modulus of patellar tendon.

2.3 Methods and materials

2.3.1 Experiment I

2.3.1.1 Patellar tendon preparation

Fresh knee joints of pigs are dissected and sold in local food markets. It was not necessary, therefore, to apply for ethics approval. Eight fresh patellar pig tendons were dissected carefully from the patella and tibia, and all soft tissues were removed from around the knee joints, leaving only patella and small tibia tuberosity (Fig. 2.1). The length of the specimens were measured by a plastic meter (Smartmax; SM-103) that was used to adjust the distance of the 2 clamps of a material testing system (MTS Synergie 200, MTS System Corporation, Ivry sur Seine Cedex, France) (Fig. 2.2). The room temperature was controlled at 25 °C.

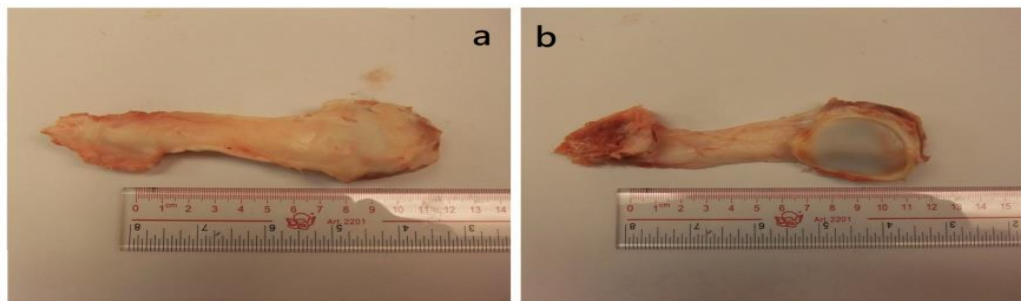


Figure 2.1 Photographs of a representative patellar tendon. (a) Anterior view for patellar tendon; (b) The length of the patellar tendon was measured by a meter.



Figure 2.2 Experiment set-up. The gel pad and transducer were put lightly on the tendon in order to capture its elasticity using Supersonic Shear Imaging.

2.3.1.2 Material Testing System

After dissection, the tangent traction modulus of patellar tendon was measured by a MTS immediately. Thus, the humidity of the specimen did not maintain. The patella and tibia of the dissected patellar tendon were connected to the 2 clamps with their fibers aligned using applied force. The tensile force (F) was applied to the 2 crossheads causing incremental displacement of the 2 clamps (d) in steps of 0.2mm at a test speed of 20mm/min until the force reached 10N. A maximum force of 10N was chosen, based on our own pilot study. When the applied force exceeded 10N, the elastic modulus reached the saturation level of SSI (300kPa). The force was captured by the load cells and the displacement of the crossheads was measured by an extensometer (MTS model 634.12F-24, MTS System Corporation, Eden Prairie, MN). Both values were displayed on-line on a computer attached to the material testing system.

2.3.1.3 Ultrasound Measurements

After the specimen was secured between the two clamps, a gel pad (ULTRAPHONIC FOCUS; Confoming gel pad; USA) was fixed onto the surface of the specimen in order to capture clear imaging. The cross-sectional area of the tendon was measured using the B-mode of the An Aixplorer® ultrasound unit (Supersonic Imaging, Aix-en-Provence, France) in conjunction with a linear-array transducer at 4-15 MHz frequency (Fig. 2.3). The ultrasound imaging is a reliable tool to assess the tendon cross sectional area (ICC=0.92) (Gellhorn and Carlson 2013). In addition, it had a higher accuracy for detecting and measuring the partial-thickness tendon tears when comparing with MRI (Lobo et al. 2013). Once a clear image of the tendon had been achieved, the shearwave

elastography mode was activated. To avoid the effect of anisotropy on the measurement, the probe was aligned with the direction of the fibres. One image was captured from each increment of 0.2mm when the tension was constant during the elastographic measurements. The image was frozen when the entire ROI was covered by the color and stored for off-line analysis.



Figure 2.3 An Aixplorer® ultrasound unit
(Supersonic Imaging, Aix-en-Provence, France)

Off-line analysis was conducted on the captured images from the SSI. A circle that delineated the region of interest (ROI) for the measurement of the elastic modulus was placed at the proximal, middle and distal parts of the SSI acquisition box on the patella tendon (Fig 2.4). The colours represented the stiffness of the tissues within the region of interest and ranged from red (hard) to blue (soft). The diameter of the ROI was determined by the width of the patellar tendon. Mean values of the elastic modulus on the patella tendon within the ROI were assessed by the built-in specific quantification

program. The elastic modulus (E) was computed from the system based on the following equation (Bercoff et al. 2004):

$$E \approx 3\mu \approx 3\rho V_s^2$$

where μ is the shear elastic modulus, density ρ is assumed to be constant (1000kg/m^3) in human soft tissue and V_s is the velocity of the shear wave propagation. Due to tendon anisotropy, the shear elastic modulus was reported (Royer et al. 2011) and the SSI provides the elastic modulus. Thus, all the values obtained using the SSI was divided by 3.

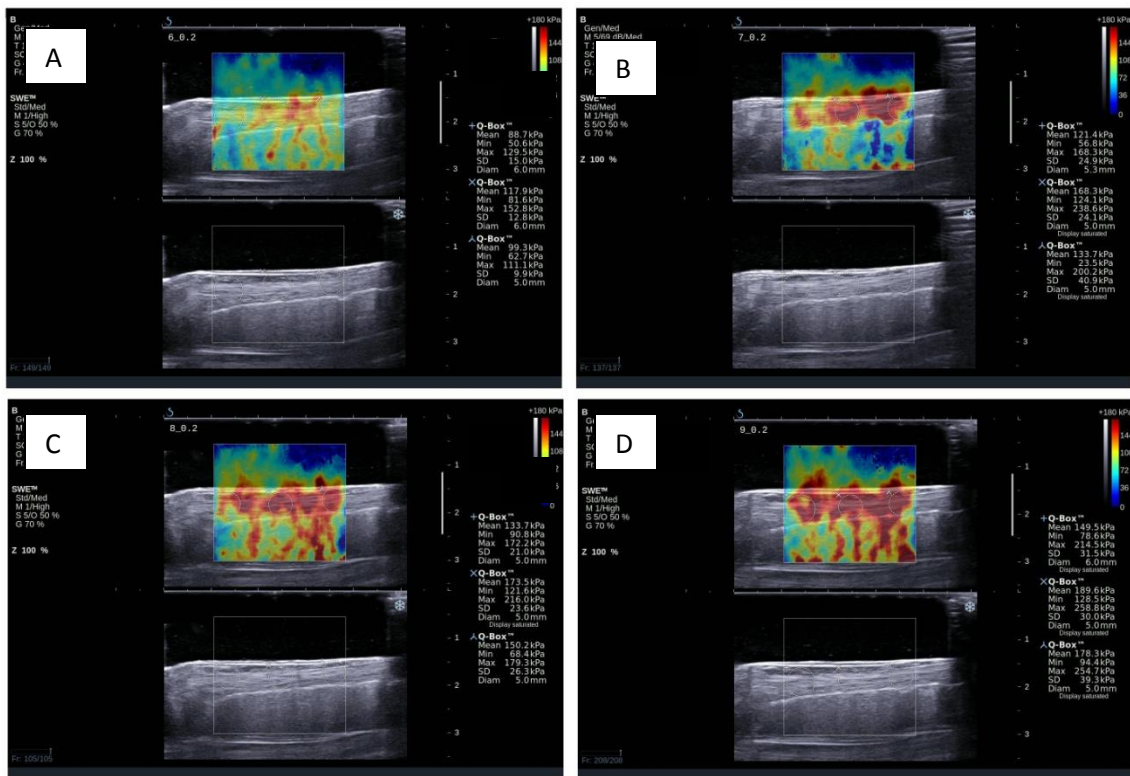


Figure 2.4 Changes of elastic modulus of pig patella tendon (PT) during increasing loading (from A to D). Upper images show the color-coded box presentation of the PT elasticity (red color represents stiffer area and green color represents softer area) with

the measurement circle representing the region of interest and its corresponding elastic modulus demonstrating under Q-Box™ on the right. The bottom images show the longitudinal grey scale sonograms of the PT to ensure the capture of clear images.

2.3.2 Experiment II

2.3.2.1 Subjects recruitment

Eleven healthy subjects (8 male, 3 female; age: 26.1 ± 3.2 years, weight: 58.7 ± 12.3 kg, height: 169.2 ± 10.0 cm) were invited to participate in this study. These subjects underwent a clinical examination to exclude patellar tendon disorders, such as patellar tendinopathy. A further exclusion criterion for participation of healthy subjects was a history of knee injury or surgery. Clinical examinations, consisting of an assessment of local tenderness over the patellar tendon and pain aggravation during single leg squatting, were performed by an experienced physiotherapist.

2.3.2.2 Imaging processing

Each participant was examined while lying supine with the knee at 30° of flexion (Bensanmoun et al. 2006). The knee was supported on a firm towel and a custom-made ankle stabilizer was used to keep the leg in neutral alignment on the coronal and transverse planes. Prior to testing, the subject was instructed to have 5-minutes of rest in a comfortable and relaxed position in order to tension on the patellar tendon was avoided (De Zordo et al. 2009). The room temperature was controlled at 25°C .

The elastic modulus of the patellar tendon was measured using an Aixplorer® ultrasound unit (Supersonic Imaging, Aix-en-Provence, France) in conjunction with a

linear-array transducer at 4-15 MHz frequency and a high frame rate (up to 20,000 frames/s). The transducer was placed longitudinally on the patellar tendon with the knee flexion of 30°. The shearwave elastography mode was then activated to measure the elastic modulus of the proximal part of the patellar tendon. The transducer was stationed on the skin, with a light pressure on top of a generous amount of coupling gel, perpendicularly on the skin's surface. The transducer was kept motionless for 8-12 seconds during the acquisition of the SSI sonogram (Kot et al. 2012). Images were frozen when the color in the region of interest was uniform and were then stored for off-line analyses. In total, 3 images were captured for the tendon on each knee.

Two operators (I and II) participated in the inter-operator investigation. Operator I had about 5 years of experience in ultrasound scanning and SSI training. Operator II was a sports physiotherapist with about 2 years of experience in ultrasound imaging as well as SSI short course training. The operators took turns to examine each subject's patellar tendon at one-hour intervals; and by Operator II with a 3-hour interval. The results were not communicated until all subjects had been examined.

A circle that delineated the region of interest (ROI) was centered at the proximal part of the tested tendon (Fig. 2.5). The diameter of ROI was defined by the thickness of the tendon, which was the distance between the superior and inferior borders of the proximal part of the patellar tendon. The mean values of the elastic modulus on the patellar tendon within the ROI were computed from the system.

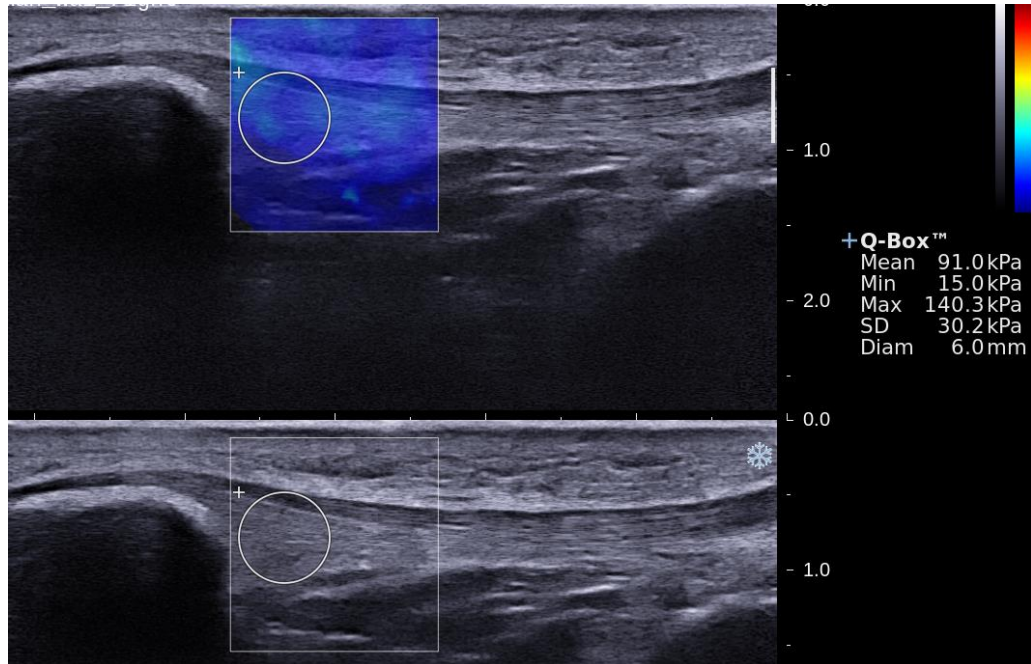


Figure 2.5 Typical example of elastic modulus measurement for the proximal patellar tendon on a healthy subject. Upper images show the color-coded box presentation of the patellar tendon elasticity (the red color represents the stiffer area and the green color represents the softer area) with the measurement circle representing the region of interest and its corresponding elastic modulus demonstrating under Q-Box™ on the right. The bottom images show the longitudinal grey scale sonograms of the patellar tendon to ensure the capture of clear images.

2.4 Statistical analyses

2.4.1 Experiment I

Data processing was conducted using the software LabVIEW 8.6 (LabVIEW Professional Development System, USA). The loads (F) and crosshead displacement (d) were recorded during testing by a computer attached to the MTS. The tensile stress (σ)

was calculated as the applied load divided by the tendon cross-sectional area (A_0) ($\sigma=F/A_0$). The tensile strain (ϵ) was calculated as displacement divided by initial length (L_0) ($\epsilon=d/L_0$). The tangent modulus was computed by a self-written programme based on the formula $E= \Delta\sigma / \Delta\epsilon$. Spearman's rank correlation tests were used to assess the level of correlation between the elastic modulus of the tendon captured from the SSI system and the tangent traction modulus calculated from the MTS.

2.4.2 Experiment II

The dependent measure for analysis was the averaged mean tendon elastic modulus from all 3 images of the patellar tendon. Both intra- and inter-operator reliability were examined using intraclass correlation coefficients (ICC). ICC(3,1) was used to determine the intra-operator reliability and ICC (2,2) was computed to examine the inter-operator reliability (Portney et al. 2009). The coefficient of variance (CV) was calculated [using the formula $CV= (\text{standard deviation}/\text{mean}) \times 100\%$]. The standard error measurement was computed (using the formula $SEM= \text{standard deviation} \times \sqrt{1-ICC}$), and minimal detectable difference was calculated (using the formula $MDD=1.96 \times SEM \times \sqrt{2}$). All reliability coefficients were interpreted as follows: below 0.499 as poor, 0.500 to 0.699 as moderate, 0.700 to 0.899 as good, and 0.900 to 1.000 as excellent (Domholdt 1993). The statistical analysis was performed using SPSS Version 17.0 for Windows (SPSS Inc, Chicago, IL).

2.5 Results

2.5.1 Experiment I

Table 2.1 shows the cross sectional area, resting length, elastic modulus and tangent traction modulus obtained from the 8 fresh pig patellar tendons. Figure 5 depicts the relationships among the SSI and MTS measurements in the specimens. Significant correlations were found between the 2 variables in all tested specimens, with the correlation coefficients ranging from 0.82 to 1.00 (all $p < 0.05$, Table 2.2, Fig. 2.6).

Table 2.1 Cross sectional area, resting length, shear elastic modulus and tangent traction modulus obtained from fresh patellar tendon.

pig	Cross sectional area (mm²)	Resting length (mm)	Mean shear elastic modulus (kPa)	Mean tangent traction modulus (kPa)
1	86.3	40.0	74.60	2736.52
2	114.0	45.0	73.33	4216.53
3	95.3	42.0	67.61	2079.83
4	71.0	65.0	75.76	5410.50
5	48.6	44.0	35.82	3736.48
6	54.0	55.0	24.34	3420.37
7	48.3	50.0	47.81	2722.72
8	46.6	40.0	52.40	6989.27

Table 2.2 Spearman's rank correlation coefficient between the shear elastic modulus and tangent traction modulus of tendon obtained from SSI and MTS, respectively.

pig	<i>Rho</i>	<i>p-value</i>
1	0.96	0.000
2	1.00	0.000
3	1.00	0.000
4	1.00	0.000
5	0.82	0.023
6	0.93	0.000
7	0.99	0.000
8	1.00	0.000

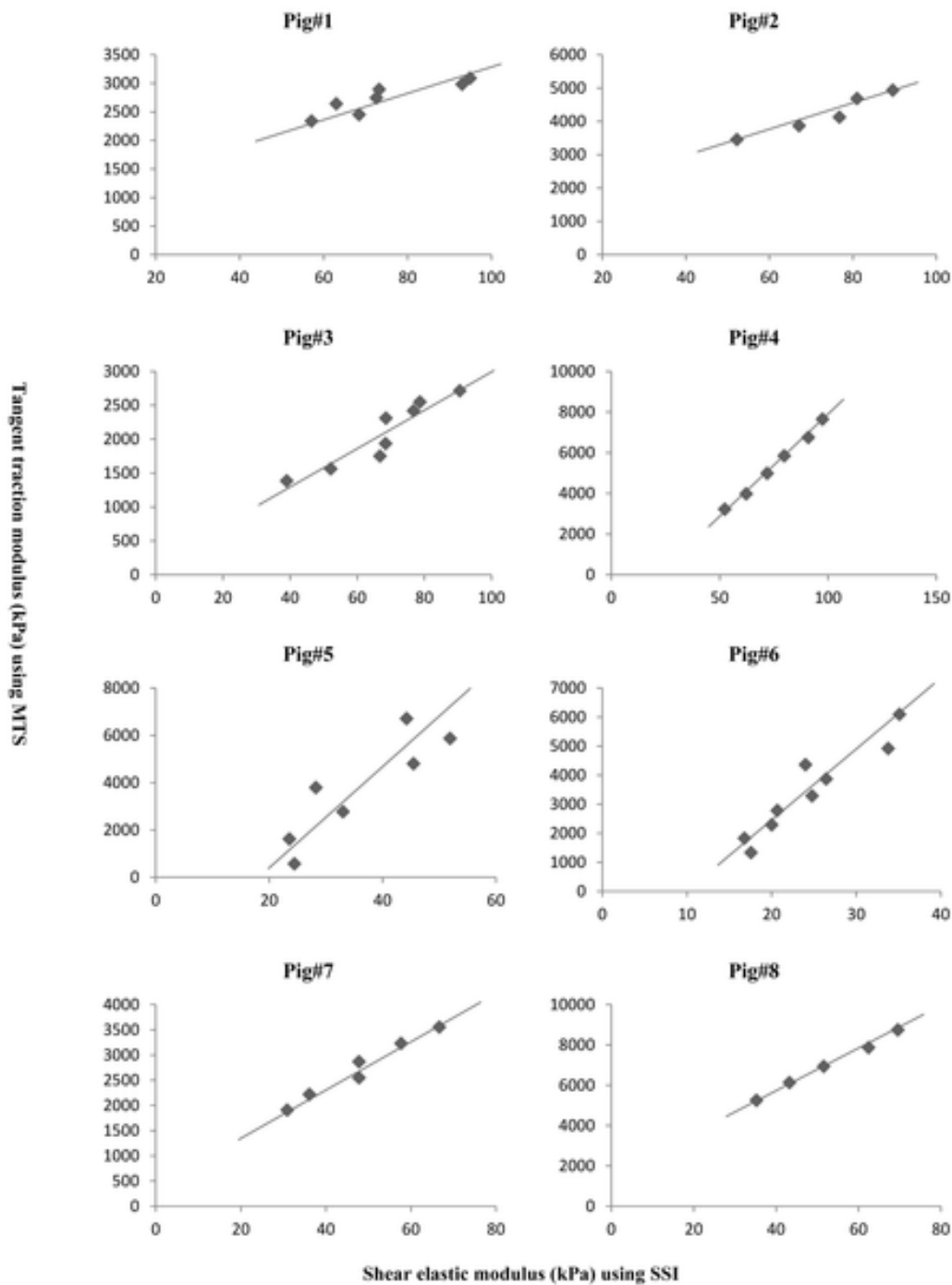


Figure 2.6 Correlations between elastic modulus of tendon captured from a Shear supersonic imaging and tangent traction modulus of tendon computed from a material testing system.

2.5.2 Experiment II

The intra and inter-operator reliabilities for the measurements of the proximal patellar tendons in the eleven healthy subjects (22 patellar tendons) were excellent. The intra-operator reliability value for ICC was 0.98 and for MDD was 4.27kPa, corresponding to an SEM of 1.54kPa and CV of 29.53%. With regard to the inter-operator reliability, the ICC value was 0.97 and the MDD value was 4.54kPa, corresponding to an SEM of 1.64 kPa and a CV of 25.89% (Table 2.3).

Table 2.3 Intra-class coefficient (ICC) values for intra and inter-operator reliabilities on the shear elastic modulus of patellar tendon.

Intra-operator reliability (Mean±SD)(kPa)					
Test 1	Test 2	ICC	95%CI	SEM	MDD
36.9±10.9	36.0±9.5	0.98	0.93-0.99	1.54kPa	4.27kPa
Inter-operator reliability(Mean±SD)(kPa)					
Operator I	Operator II	ICC	95%CI	SEM	MMD
36.9±10.9	36.7±9.5	0.97	0.93-0.98	1.64kPa	4.54kPa

Abbreviations: ICC=intra correlation coefficient; CI=confidence index ;SEM=Standard error measurement ;MDD=minimal detectable difference

2.6 Discussion

Significant correlations were found between the elastic modulus on the patellar tendon captured from the SSI and the tangent traction modulus computed from the MTS. Excellent intra and inter-operator reliabilities were obtained when the SSI was performed on the healthy patellar tendons.

High correlations were observed between the elastic modulus measurement using the SSI and the tangent traction modulus obtained from the MTS of the 8 pig patellar

tendons. We also observed large differences in the slopes of different specimens. Hence, there was no single transformation from the elastic modulus to the tangent modulus that covered all specimens. We postulate that such differences might relate to the following factors. The MTS is known to have several inherent limitations. The stretching of the clamped fibrous tissues is associated with some slippage of inner fibres (Maganaris et al. 2002). In this study, the bones (patella/ tibia tuberosity) were fixed tightly on the MTS. Some of the stretching force imposed from the MTS might have been taken up at the system-bone interface, which might have been different on each tendon. Soft tissues (fat/fascia) around the tendons had to be removed by hand. Any remaining tissues would have contributed to some of the loads. Despite great care being exercised to clear the tissue around the tendons, the amount of tissue remaining might have been different on each tendon. The tangent modulus computed from the MTS might thereby have been influenced by the above two technical issues to different extents on the tested tendons. On the other hand, intrinsic factors such as age and gender can contribute to the viscoelastic properties of the tendon. The viscoelastic properties of the patellar tendon may alter structurally with age, for example changes in collagen content. In an animal model, Haut et al (Haut et al. 1992) reported a decrease in the collagen content of the patellar tendon with age. Similar observations were also found in human studies (Tkaczuk 1968; Noyes and Grood 1976). Gender may also affect the viscoelastic properties of the patellar tendon. Kubo (Kubo et al. 2003) reported that the stiffness of the medial gastrocnemius tendon was significantly higher in males (approximately 37%) than in females. Note that, in this study, the elastic modulus was calculated based on the assumption that the density of the medium was a constant. This, however, may have been

affected by the age or gender of the testing tendons, as we did not control for either of these.

To the best of our knowledge, the present investigation is the first to report the relationship between the modulus elastic using the SSI and the tangent traction modulus from the MTS measurement on tendons. All of the reported studies compared the muscle elastic modulus with muscle activities based on electromyography studies. Nordez's was the first group to report that the elastic modulus of the biceps muscles of 6 healthy subjects was related strongly to the EMG activity level during muscle isometric contraction (Nordez and Hug 2010). Significant linear relationships between the elastic modulus and the individual muscle force were also reported on the small hand muscles such as the abductor digiti minimi and first dorsal interosseous (Bouillard et al. 2011), indicating that the elastic modulus could be used to estimate tension inside tissues. In addition, Maïsetti et al. (2012) reported a significant correlation between the elastic modulus and medial gastrocnemius muscle force during passive stretching. The correlations reported in the present study were possible only because both moduli were increased when the tension was increased.

The ultrasonography method has been used extensively over the last 20 years to assess the elastic properties of tendons. The elastic properties of the tibialis anterior and gastrocnemius tendons have been measured by ultrasound imaging as a method of estimating tendon-aponeurosis elongation during the tensile loading induced by the contraction of the in-series muscle (Maganaris et al. 2002). Hansen et al. (2006) reported that the method to assess the elastic properties of human patellar tendon is reliable using ultrasonography with EMG during quadriceps muscle isometric contraction. The EMG

has been used simply by some authors to correct force measurements from the antagonist contribution. Although this is a useful method to evaluate the elastic properties of the tendon, there are some limitations, such as complex techniques (transducer fixation technique), knee joint movement control, time consumption, complicated data analysis procedures and dependence on the muscle contraction (Hansen et al. 2006). Due to above the barriers, it is difficult to apply this method in the hospital and clinic to estimate the elastic properties of tendons. The SSI has overcome these limitations in the elastic properties measurement of the tendon, and this study has supported it as a relatively convenient method of measuring the elastic property of the patellar tendon.

Experiment II evaluated the intra and inter-operator reliability in obtaining the SSI elastic modulus measurements of the healthy participants' proximal patellar tendons at the rest position within-day. If subjects with tendon disorders are assessed several times by different examiners, it is important to know the intra and inter-operator reliabilities. The results of this study have demonstrated that the SSI of the proximal patellar tendon has excellent intra and inter-operator reliabilities in healthy subjects. The SSI can be used to assess disease progression and the efficacy of intervention on patellar tendons when evaluations have to be conducted at different time points.

It has been reported that the SSI is a reliable method for evaluating the elastic properties of muscles. Lacourpaille (Lacourpaille et al. 2012) reported that the ICC value of intra- and inter-operator reliability among various muscles (gastrocnemius medialis/tibialis anterior/rectus femoris/biceps brachii/ triceps brachii/ vastus lateralis) during resting status ranged from 0.81 to 0.95 and 0.42 to 0.94, respectively. Another study revealed that the intra-operator reliability of the elastic properties of biceps brachii

during 3% and 7% maximal EMG activity were good (3%, ICC=0.89; 7%, ICC=0.94) (Kubo et al. 2003). Our intra and inter-reliabilities results of 0.98 and 0.97 respectively are higher than those reported from these studies of muscles. One of the reasons for this may be related to the different structures of the muscle and tendon. A tendon consists of parallel collagen fibers, and it is easier to align and re-align the US probe with the tendon than with the muscle fibers, resulting in higher reliability.

Drakonaki et al (2009) obtained moderate to good intra and inter-operator reliability in assessing the stiffness of Achilles tendons using real-time freehand ultrasound elastography which depends on compressive force. In their study, 25 healthy subjects were recruited for the assessment of tendon stiffness at the middle third of the free tendon and the middle part between the myotendinous junction and the calcaneal insertion. The intra and inter-operator reliabilities ranged from moderate to good (0.51-0.78). One of the major differences between the SSI and real-time freehand ultrasound elastography is that the mechanical vibration is induced automatically by using a radiation force of ultrasound beams (Bercoff et al. 2004). Thus, the SSI technique does not depend on external force from operators. This may be one of the main explanations why the SSI technique is more reliable than real-time free-hand ultrasound elastography.

In addition, our study calculated the MDD, which can provide a value to reflect a real change as a reference for future study. In terms of our findings, the measurement of the elastic modulus of the patellar tendon should be greater than 4.27kPa (the same operator) and 4.54kPa (different operator) to reflect real changes with retested measurements.

There are some advantages of the SSI technique when compared with other methods to evaluate the tendon elastic properties. First, it is a reliable and convenient technique to assess the elastic properties of the tendon. In the present study, the time required for scanning 2 tendons lasted for 5-8 minutes. Second, the operation of the machine can be learnt by a novice. Although Operator I (2years) and Operator II (5years) had different lengths of experience with the ultrasound scanning technique, the findings from the present study have demonstrated good intra and inter-reliabilities of the SSI measurement on the tendon elastic modulus, which indicates that the results could not have been influenced by the operator's experience. Finally, the SSI can be used to evaluate tendon elastic properties that are not affected by the presence of pain. The conventional approach, based on ultrasonography and the EMG, induces an increase in the tensile force on the tested tendon that might cause pain on a tendon with pathologies. These advantages of the SSI make it a promising clinical tool to follow disease progression and enhance the efficacy of different interventions.

In this study, the mean elastic modulus on the healthy patellar tendons ranged from 36.0 to 36.9kPa. The results from the present study were higher than those reported by Kot et al. (2012). The mean elastic modulus on healthy patellar tendons being reported were 23 to 24 kPa. Such discrepancies might relate to the different method of defining the ROI. In Kot's study, the ROI was pre-determined (2mm, 3mm or 4mm). In the present study, we adopted the approach used by Nordez and Hug (2010). The diameter of the ROI was defined by the thickness of the tendon, which was the distance between the superior and inferior borders of the proximal part of the patellar tendon. Note that the tendon thicknesses in this study ranged from 3 to 7mm and different portions of the

patellar tendon contain different percentages of collagen fibers (Maïsetti et al. 2012). Our approach, thereby, included the whole rather than portion of the tendon.

2.7 Conclusion

The present study has indicated that the elastic modulus on the patellar tendon measured from the SSI is related closely to the tangent traction modulus calculated from the MTS. The *in-vivo* measurement has illustrated excellent reliability of this tool. The SSI can be applied to evaluate the elastic properties of a healthy patellar tendon. The diagnostic role of this technique will be investigated by assessing the shear elastic modulus of normal and pathological tendons, as well as to monitor disease progression and the efficacy of intervention on individuals with tendon disorders.

CHAPTER 3

ELASTIC PROPERTIES OF THE PROXIMAL PATELLAR TENDON: EFFECTS OF AGE, BMI, LEG DOMINANCE, AND SPORTS PARTICIPATION

3.1 Abstract

Purpose: To compare the elastic properties of the patellar tendon among sedentary subjects, volleyball players, and basketball players, and to examine the correlations between the elastic properties of the proximal patellar tendon and the age, body mass index, and training intensity of players.

Study design: Cross-sectional observational study.

Methods: Fifty healthy subjects (20 sedentary, 15 volleyball players, and 15 basketball players) aged between 18 and 35 were recruited. SSI was used to measure the elastic properties of the proximal patellar tendons in the dominant and non-dominant knees.

Results: The volleyball players had the lowest tendon shear elastic modulus compared with the basketball players (by 24.9%, $p<0.05$) and sedentary subjects (by 42.3%, $p<0.05$). The basketball players had significantly lower tendon elastic modulus compared to the sedentary group (by 23.1%, $p<0.05$). No significant side-to-side difference in the tendon shear elastic modulus was detected ($p>0.05$). A positive correlation was detected between the patellar tendon shear elastic modulus and age in the volleyball group (partial correlation $r=0.53$; $p<0.05$).

Conclusions: The elastic properties of the patellar tendon can be adapted with sports participation. The magnitude of the tendon adaptation appeared to be greater for the volleyball than the basketball players. The volleyball players, who had greater demands in terms of jump-landing activities and hence more mechanical loading on the patellar tendon, demonstrated greater tendon compliance than the basketball players. Age was the

only anthropometric factor related to the elastic properties of patellar tendon in the volleyball players.

Key words: Elastic properties; proximal patellar tendon; sports activities

3.2 Introduction

The patellar tendon is an energy-storing tendon responsible for transmitting muscle-derived forces to produce joint movement (Alexander 2002; Magnusson et al. 2003). The tendon also acts like a spring in storing elastic energy for energy-efficient movements (Arampatzis et al. 2006; Kubo et al. 2010), and functions as a “mechanical buffer” in reducing the rate of force transmission to the muscle to prevent muscle injury (Konow et al. 2012; Magnusson et al. 2003). A rigid tendon is better able to transmit the muscle-derived force to the bone, whereas an elastic tendon can store more energy for energy-efficient movements.

Physical activities such as strength training and habitual loading can induce mechanical loading on the tendon and alter its mechanical properties. Stiffness of the patellar tendon was found to have increased by 65% after a 14-week isometric training program (Reeves et al. 2003). The elastic properties of the patellar tendon were assessed by ultrasound imaging with a dynamometer during muscle contraction. Similarly, three-months’ of isometric training of knee extensors with long duration contraction was found to increase tendon stiffness by 36.4% (Kubo et al. 2010). No significant change on tendon elastic properties was observed with short duration contraction. In contrast, a single bout of hopping exercise did not induce significant changes in the elastic properties of the patellar tendon (Peltonen et al. 2010). Collectively, these findings suggest that 12 to 14 weeks’ isometric exercise training increase tendon stiffness. The duration of contraction may also affect the loading on tendon.

Only one study has investigated the effect of habitual loading on the elastic properties of the patellar tendon. Westh et al. (2008) assessed the elastic properties of patellar tendon in runners and non-runners. Although the patellar tendon shown greater compliance in the runner compared to the non-runners (by 14.8%), the difference was not statistically significant level. These results from this study needs to be treated with caution because of the small sample size used in the study (only 10 subjects were recruited in each team). To better understand the loading effects of habitual exercise, a study with a larger sample size and in sports with more loading on the patellar tendon, such as the jumping sports, is needed.

Volleyball and basketball are two common jumping sports that have different physical demands (Kollias et al. 2004; Laffaye et al. 2007). During training and competition, volleyball players take more frequent vertical jumps and land with greater knee flexion (Kollias et al. 2004; Laffaye et al. 2007) than basketball players. The volleyball players were also found to have greater jumping height (Laffaye et al. 2007). It is likely that the elastic properties of the patellar tendon adapt differently to the sport-specific loading from volleyball and basketball training. Knowledge of the tendon adaptation to each sport would advance our understanding of the relationship between mechanical loading and tendon adaptation. This information is essential for designing sport-targeted injury prevention programs for strain-induced injuries, such as tendinopathy.

Aside from mechanical loading, age-related changes in the diameter of the collagen fibers may contribute to the change in tendon stiffness (Sargon et al. 2005; Strocchi et al. 1991). Kubo et al. (2007) detected a decrease in the stiffness of the

Achilles tendon in older adults compared to younger adults. Stenroth et al. (2012) also found that the Achilles tendons of the older subjects were on average 32% less stiff than those in the younger group. When measuring the elastic properties of the patellar tendon, Coupe et al. (2009) reported a similar magnitude of change between old and young subjects. The older subjects had 29% less stiff than the younger subjects. However, Mian et al. (2007) found no significant difference in the elastic properties of the muscle-tendon of the gastrocnemius medialis between young and old adults. Similarly, Carroll et al. (2008) reported that there were no significant differences in the elastic properties of the patellar tendon in younger and older adults. Thus, the existing findings on the effects of age on the elastic properties of the human patellar tendon are inconclusive.

The aim of this study was to investigate the changes in the tendon elastic properties of the proximal patellar tendon as a result of sports participation. The tendon shear elastic moduli of sedentary subjects, volleyball players, and basketball players were compared in the dominant and non-dominant legs. Possible correlations between the tendon shear elastic modulus, age, BMI, and training intensity were explored. We hypothesized that volleyball players have better tendon compliance than basketball players and there would be correlation between tendon shear elastic modulus, age and body mass.

3.3 Material and methods

3.3.1 Subject recruitment

A convenience sample of 50 healthy male subjects was recruited from the volleyball and basketball teams of local universities. The participants comprised 20 sedentary subjects, 15 volleyball players, and 15 basketball players. All of the subjects

were interviewed on their past and current physical activity. The inclusion criteria for healthy players were as follows. They should train regularly for more than four hours per week, be free from injury or discomfort in the knee, and not be taking any steroids at the time of the study. Subjects were excluded if they had had any surgery on the lower limbs. The sedentary subjects were not involved in any regular exercise and sports training.

3.3.2 Experimental procedure

The study was conducted at the Sports Training Centre at the Hong Kong Polytechnic University. The body weight and height of all subjects were measured. The level of training intensity (training hours/week) was self-reported. The dominant leg was determined by kicking a ball (Lenskjold et al. 2013).

3.3.3 Measurement on tendon elastic properties

The tendon elastic properties were measured using supersonic shearwave imaging (SSI) technology with an Aixplorer[®] ultrasound unit (Supersonic Imaging, Aix-en-Provence, France) equipped with a 4-15 MHz linear-array transducer. The set-up and procedure are described in Chapter 2. In brief, the musculoskeletal acquisition mode was used to measure the shear elastic modulus of the patellar tendon with the temporal averaging (persistence) and spatial smoothing set to medium and six, respectively. The range of the color scale was pre-set by the manufacturer at 0-600 kPa. The angle between the ultrasonic beam and tendon fiber orientation would influence the results of tendon stiffness. In order to minimize the effect of anisotropy, they suggested that the transducers will be paralleled to the tendon fibers during measuring the stiffness of tendon (Brum et al. 2014; DeWall et al. 2014).

Scanning of patellar tendon was performed with the subject in supine lying, with the knee in 30° of flexion (Zhang and Fu 2013). The transducer was positioned at the inferior pole of the patella and aligned with the patellar tendon. The transducer was stationed on the skin with light pressure on top of a generous amount of coupling gel, perpendicularly on the surface of the skin. The transducer was kept stationary for 8-12 seconds during the acquisition of the SSI sonogram (Fig. 3.1). A total of 3 images were captured for the tendon on each knee for off-line analysis.

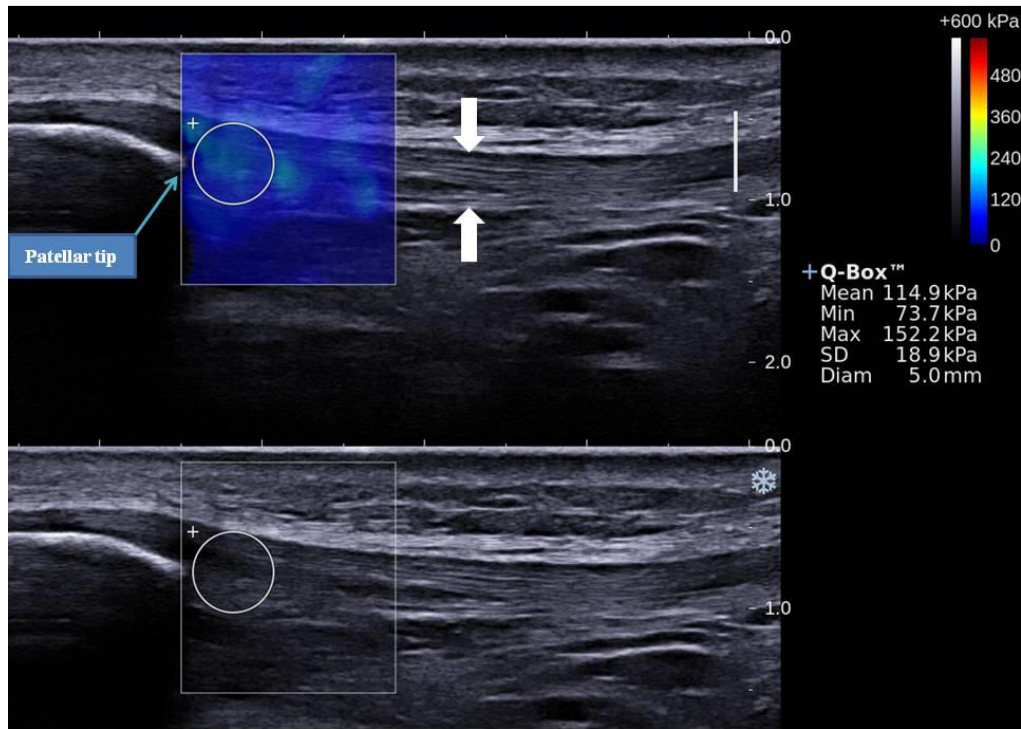


Figure 3.1 Representative in vivo image of a proximal patellar tendon. The patellar tip, superficial and deep border of patellar tendon was identified with arrows. The size of Q-box™ was based on the superficial and deep border of patellar tendon. The Q-box™ was located in the proximal part of patellar tendon. Local tendon elastic modulus is estimated at each pixel within the Q-box™ and represented by the color map.

3.3.4 Data Reduction

Off-line analysis was conducted according to the procedures described in last chapter. In brief, the built-in region of interest (ROI) rectangular box of 13.5mm×12.5mm was placed distal to the apex of the patellar with the patellar tendon located within its centre part. The Q-Box™ indicated a semitransparent color map was centered at the proximal part of the patellar tendon with consistent images and at about 5mm from the apex of the patella. Young's modulus within the Q-Box™ were computed and displayed in kPa at the right bottom corner of the computer screen. The mean tendon shear elastic modulus was calculated by dividing the mean Young's modulus generated from the system by 3 (Royer et al. 2011). The patellar tendon shear elastic modulus has excellent test-retest reliability (with ICC=0.98) (Zhang and Fu 2013).

3.3.5 Statistical analysis

Descriptive data such as mean±SD were calculated. The Shapiro-Wilk tests were used to examine the normality distribution of variables. One-way analysis of variance (ANOVA) was used to compare the age, height, weight, BMI, training intensity between sedentary, basketball and volleyball players. Bonferroni post hoc analysis was used to examine group differences. Two-way analysis of covariance tests were used to compare the tendon shear elastic modulus with group (sedentary, volleyball, basketball) and leg (dominant and non-dominant) as independent factors with demography and training variables having significant group difference as covariates. If between-group difference was identified, Bonferroni post hoc analysis was used to examine group differences. Partial correlation tests were used to examine the correlations between tendon shear

elastic modulus with anthropometric characteristics and training intensity controlled by leg dominance in each group. SPSS version 17.0 (SPSS Inc, Chicago, IL) was used to perform the statistical analyses. The level of significance was set at $p < 0.05$.

3.4 Results

3.4.1 Subjects characteristics

Twenty sedentary subjects, 15 volleyball players, and 15 basketball players participated in this study. Significant group differences were detected on age and BMI. The basketball players were older and had greater BMIs compared with the subjects in the sedentary and volleyball groups. There were no significant differences in age and BMI between the volleyball and sedentary groups. There was no significant difference in the hours of training between the volleyball and basketball groups (Table 3.1).

Table 3.1 Characteristics of subjects in the study

Variables	Sedentary subjects (n=20)	Basketball players (n=15)	Volleyball players (n=15)	P value		
				S vs B	S vs V	V vs B
Age(y)	24.0±3.4	26.3±3.9	22.2±4.6	0.271	0.566	0.019
Weight(kg)	58.6±6.8	78.5±6.8	68.3±7.4	0.000	0.001	0.001
Height(cm)	170.8±5.8	184.9±5.7	181.0±5.8	0.000	0.000	0.218
BMI (kg/m ²)	20.1±1.6	23.0±1.6	20.8±1.8	0.000	0.567	0.002
Training hours /w	0.6±0.5	8.8±3.9	8.9±4.0	0.000	0.000	0.851
Training years		10.0±5.4	7.1 ±1.9			0.156

Abbreviations: S=Sedentary subjects; B=Basketball players; V=Volleyball players

3.4.2 Comparison of the tendon elastic properties of the sedentary, volleyball players, and basketball players

Significant differences in tendon shear elastic modulus were found between 3 groups. The tendon shear elastic modulus of the sedentary, basketball, and volleyball

groups was found to be 40.2 ± 10.6 kPa, 30.9 ± 6.1 kPa and 23.2 ± 8.8 kPa, respectively (Tables 3.2). Sedentary subjects had a significantly higher shear elastic modulus than volleyball players (by 42.6%; $p=0.000$) and basketball players (by 18.6%; $p=0.003$) (Fig 3.2). In addition, the basketball players had a higher shear elastic modulus than the volleyball players (by 24.9%; $p=0.020$) (Fig.3.2). No significant side-to-side effects were detected (Table 3.3; Fig. 3.3).

Table 3.2 Comparisons of patellar tendon shear elastic modulus between three groups

shear elastic modulus (kPa)	Sedentary subjects (n=20)	Basketball players (n=15)	Volleyball players (n=15)	P value		
				S vs B	S vs V	V vs B
Patellar tendon	40.2 ± 10.6	30.9 ± 6.1	23.2 ± 8.8	0.003	0.000	0.020

Abbreviations: S=Sedentary subjects; B=Basketball players; V=Volleyball players

Table 3.3 Side-to-side comparisons of the shear elastic modulus of the proximal patellar tendon

Variables	Shear elastic modulus (kPa)		P value
	Dominant side	Non-dominant side	
Sedentary subjects (n=20)	39.1 ± 10.1	40.2 ± 10.6	0.382
Basketball players (n=15)	33.8 ± 8.7	30.9 ± 6.0	0.139
Volleyball players (n=15)	22.6 ± 10.6	23.0 ± 8.8	0.678

Results are presented in terms of mean \pm SD

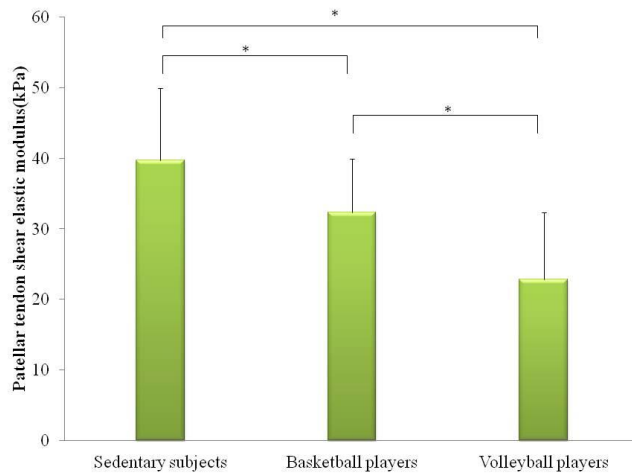


Figure 3.2 Bar graph illustrating the shear elastic modulus of proximal patellar tendon for sedentary subjects, basketball players and volleyball players ($*p<0.05$)

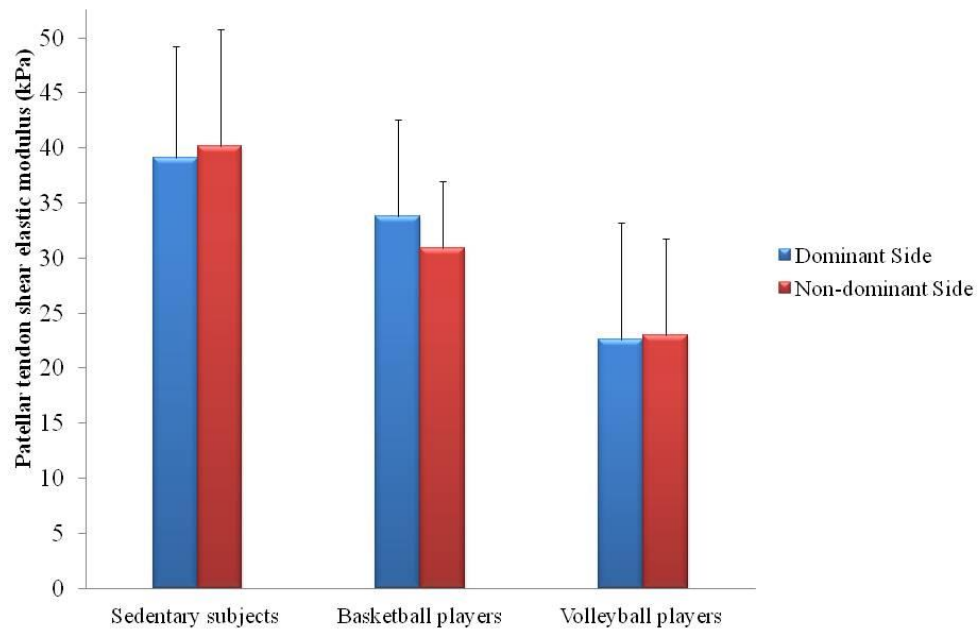


Figure 3.3 Mean shear elastic modulus of proximal patellar tendon for sedentary subjects, basketball players and volleyball players. No significant differences were detected between dominant and non-dominant side.

3.4.3 The relationships between tendon shear elastic modulus with anthropometric characteristics and training intensity in each group

Table 3.4 shows the relationships between tendon shear elastic modulus, and anthropometric characteristics and training intensity of each group. A positive correlation was found between the tendon shear elastic modulus and age of the volleyball players ($r=0.53$; $p<0.05$). No significant correlation was found between patellar tendon shear elastic modulus and other factors (weight/height/BMI/training intensity) in any of the groups.

Table 3.4 Partial correlations coefficient tests were used to examine the correlation between tendon shear elastic modulus with anthropometric characteristics and training intensity

Variables	Sedentary subjects (n=20)	Basketball players (n=15)	Volleyball players (n=15)
Age	0.07	-0.21	0.53*
Weight	-0.09	0.01	-0.03
Height	-0.28	-0.26	0.19
BMI(kg/m ²)	0.12	0.25	-0.19
Training hours/w	0.11	0.03	-0.19

* $p<0.05$; BMI=body mass index.

3.5 Discussion

This study aimed to assess the effect of habitual loading on tendon elastic properties using volleyball and basketball players as examples. The effects of mechanical loading on the tendon elastic properties were explored in relation to the type of sport, age, BMI, leg dominance, and training intensity of the subjects. The primary findings indicate that the volleyball and basketball players showed better compliance on the patellar tendon. This enhancement was found to be greater in the volleyball players than the basketball

players. Only the age of male volleyball players was found to be related to tendon elasticity.

An increase in tendon elasticity at the proximal patellar tendon was observed in the male volleyball and basketball players compared with the sedentary subjects. Only one study has investigated the effects of habitual loading on the elastic properties of the patellar tendon (Westh et al. 2008). Ten runners were found to have a 14.8% increase tendon compliance compared with 10 non-runners. However, the difference was not statistically significant, partly due to the small number of subjects. Fifty subjects participated in the present study and comparison was made between the subjects who participated and did not participate in jumping sports. Jumping sports involve repetitive jumping activities, which impose more loading on landing than running on the patellar tendon. In addition, the SSI technique was used to directly measure the tendon elastic properties. The ultrasound probe was located at the proximal portion of the patellar tendon that was found to have greater mechanical loading than other parts of the tendon. Kongsgaard et al. (2007) reported that 12 weeks of resistance knee extension training increased the proximal and distal cross-sectional area, but not the mid-region of the patellar tendon. Based on this observation, the measurements of the tendon elastic properties were taken at a region with high loading and where pathological changes would occur, i.e. the proximal portion of the patellar tendon (Kulig et al. 2013). Indeed, the results of this study indicated greater tendon elasticity at the proximal portion in male volleyball and basketball players compared with sedentary individuals.

Tendon elastic properties are essential for jumping performance. Walshe and Wilson (1997) found a positive relationship between tendon elasticity and

countermovement jump height. The authors suggested that a tendon with more compliance can store and release more kinetic energy for subsequent jumps. In a later study, Kubo et al. (1999) observed that greater elasticity at the musculotendinous junction of the vastus lateralis was related to higher vertical jump height. Kubo et al. (2007) later reported a significant relationship between the elasticity of the Achilles tendon and the height of countermovement and drop jumps. Increased tendon elasticity at the proximal patellar tendon is therefore beneficial to the jump performance of athletes such as volleyball and basketball players. However, the present study did not confirm whether the differences in the tendon elastic properties of the sedentary and jumping athletes were related to sports adaptation or that individuals who could jump higher because of their better tendon elasticity chose to participate in jumping sports. Further research is needed to verify the cause-effect relationship.

The magnitude of the mechanical loading associated with basketball and volleyball appears to be different. During training and competition, volleyball players perform more vertical jumps and undergo greater knee flexion than basketball players. The findings from this study provided evidence that volleyball players have 24.9% greater patellar tendon elasticity than basketball players. Related to the increase in tendon elasticity, Laffaye et al. (2007) found that volleyball players have a greater jumping height than basketball players. Further research is needed to directly examine the correlation between the tendon elastic properties and jumping height. The findings would provide evidence for performance enhancement programs for jumping athletes.

Side-to-side differences in elastic properties of proximal patellar tendon

No significant differences were found in the elastic properties of the proximal patellar tendons of the dominant and non-dominant legs of sedentary subjects, basketball players, and volleyball players. Coupe et al. (2008) also found that the elastic moduli of the patellar tendons on the dominant and non-dominant legs were similar in players from badminton and fencing teams. Together, these findings suggest that there are no side-to-side differences in the patellar tendon elasticity of sedentary subjects and athletes. Similarly, Holmes and Alderink (1984) found no significant side-to-side differences in the strength of the quadriceps muscles. Burgess et al. (2007) observed that the elastic properties of the tendon were partly affected by the muscle force output, although they found no significant side-to-side differences in the strength of the quadriceps muscles, thereby supporting the finding of no significant side-to-side differences in patellar tendon elasticity. These findings suggest that an internal comparison could be conducted in patients with unilateral problems, such as patellar tendinopathy.

Although the elastic properties of the proximal patellar tendon were not related to weight, height, BMI, or training intensity, they were positively correlated with the age of the volleyball players. This finding indicated that the older volleyball players had stiffer proximal patellar tendons compared with the younger players with similar training intensity. In line with this, the digital flexors and extensors of the tendons in mature pigs were found to be stiffer than in newborn pigs (Shadwick 1990). In a human study, 7 older men were found to have stiffer (by 6.9%) patellar tendons compared to 16 young men (Carroll et al. 2008). However, the difference was not statistically significant. The age-related decline in tendon compliance may be due to the decreased water content (Danielsen et al. 1988), increased fibril diameter (Parry et al. 1978), and cross-link

increments (Reiser et al. 1987). However, no significant relationships between age and tendon elastic properties were observed in the sedentary and basketball players. Hence, the age-related adaptation of the tendon elastic properties was only found in the volleyball players with high mechanical loading. In this connection, our subjects had age ranged from 18 to 35 and there was significant difference in age between volleyball and basketball players. Hence, group comparisons were conducted with age as a confounding factor.

3.6 Limitations

The elastic properties of the proximal patellar tendon were assessed because the proximal patellar tendon is highly sensitive to mechanical loading (Kongsgaard et al. 2007) and the pathology of the patellar tendon mostly occurs in the proximal region (Kulig et al. 2013). The finding of an increase in tendon elasticity does not represent changes in other parts of the tendon. Second, only male subjects were recruited in the study, therefore the results cannot be generalized to the female population. Further research is needed to examine the influence of habitual loading on the tendon elastic properties of female subjects. Lastly, the number of hours of training was used as the measure of loading induced by sports participation. Other loadings, such as the loading from conditioning, weight training sessions, and participation in other sports, need to be examined to gain a better understanding of the effects of the training intensity or training load on the athletes. Moreover, the finding that training intensity is not related to tendon elasticity needs to be treated with caution. Further prospective studies are needed to assess the effects of sports on tendon elasticity while controlling for the training load.

3.7 Conclusions

In this study, the proximal patellar tendon was found to be more compliant in males who participated in volleyball and basketball for more than four hours per week than in sedentary subjects. Moreover, the volleyball players were found to have greater compliance than the basketball players. These findings suggest that habitual loading may induce adaptation of patellar tendon elasticity and that the adaptation is sport-specific. Prospective studies are needed to confirm the effects of habitual loading from sport participation while controlling for training intensity. In addition, age appeared to reduce tendon compliance in the volleyball players. However, no side-to-side dissymmetry in the tendon elastic properties of the proximal patellar tendon was observed in the healthy sedentary subjects or the athletes. This suggests that volleyball players may experience more impact loading on the patellar tendon and therefore be more prone to strain-induced tendinopathy.

CHAPTER 4

HIP STRENGTH IN MALE ATHLETES WITH AND WITHOUT PATELLAR TENDINOPATHY AND ITS ASSOCIATION WITH THE ELASTIC PROPERTIES OF THE PATELLAR TENDON

4.1 Abstract

Objectives: To compare the muscle strength of the hip abductors and external rotators in male athletes diagnosed with patellar tendinopathy (PT) with those of healthy controls and to explore the association between patellar tendon stiffness and hip strength.

Study design: Cross-sectional observational study

Methods: Sixty-six male athletes (mean age of 21.1 ± 4.4 years) participated in this study, including 33 subjects diagnosed with PT for more than three months. The muscle strength of the hip abductors and external rotators was quantified using a hand-held dynamometer. The elastic properties of the patellar tendon and the vastus lateralis (VL) and rectus lateralis (RF) muscles were assessed using supersonic shearwave imaging technology.

Results: No significant side-to-side differences were detected in the hip muscle strength and elastic shear modulus on the patellar tendon and the VL and RF muscles in the control group (paired-*t*-tests, all $p > 0.05$). MANOVA tests were used to compare the measurements on the painful side for the subjects with PT and on the dominant leg for the controls. The normalized hip muscle strength in the PT group was found to be significantly weaker (all $p < 0.05$) when compared with the healthy controls. In the subjects with PT, the normalized muscle strength was lower by 18.2% ($p = 0.000$) and by 11.2% ($p = 0.007$) in the hip abductors and external rotators, respectively, when compared with the healthy controls. Significant increases in the elastic shear modulus of the patellar tendon from by 48.0% ($p = 0.000$) and the VL by 26.5% ($p = 0.000$) were observed in the subjects with PT when compared with the healthy controls. Significant correlations were detected between the tendon shear elastic modulus and normalized muscle strength of the

hip abductors ($r=-0.49$; $p=0.004$) and external rotators ($r=-0.40$; $p=0.025$) in the volleyball players; and between the tendon shear elastic modulus and normalized muscle strength of the hip abductors ($r=-0.55$; $p=0.001$) in the basketball players. Normalized muscle strength of the hip abductors of 35.7% and external rotators strength of 17.9% were found to differentiate subjects with and without PT with a sensitivity of 65.7 % and 68.6% and a specificity of 90.0% and 70.0%, respectively. The Youden's index for the shear elastic modulus of the patellar tendon was 39.4 kPa with a sensitivity of 75.0% and a specificity of 93.3% and the index for the shear elastic modulus of the VL muscle was 4.2 kPa with a sensitivity of 86.1% and a specificity of 90.0%.

Conclusions: Athletes with PT have less muscle strength in the hip abductors and external rotators muscles. Weakness in the hip muscles is associated with reduced elasticity in the patellar tendon. These findings suggest that strengthening programs targeting the hip abductors and external rotators are recommended for the prevention of patellar tendinopathy in volleyball and basketball players.

4.2 Introduction

Overloading has been suggested to be a causative factor in the development of patellar tendinopathy (Lian et al. 1996). The risk factors of tendon overloading are considered to be multifactorial. Extrinsic factors, such as training frequency, training duration, training intensity (Neely et al. 1998), and the training surface (Lian et al. 2003; Torstensen et al. 1994), have been investigated and reported. Intrinsic factors such as muscle weakness and tightness also induce excessive mechanical loading on the patellar tendon.

Sporting activities, such as the rapid deceleration from landing, place large loads on the lower extremities. The muscle-tendon units of the hip, knee, and ankle joints act to dissipate the kinetic energy on landing (Fredberg et al. 1999). Decker et al. (2003) reported that the knee extensor was the primary shock absorber during landing among male and female subjects, and the hip muscles were the second largest contributor to energy absorption during landing among male subjects. Powers (2010) demonstrated that reducing demand on the hip muscles during landing would increase the demand on the knee extensors, and this maneuver may increase the knee joint flexion moment and loading on the knee joint. Therefore, weakness in the hip muscles may impose excessive loading on the patellar tendon. In addition, altered landing kinematics due to inadequate hip control may contribute to the excessive loading on the knee joint and increased risk of PT. Edward et al. (2010) observed increased hip adduction and internal rotation and knee abduction and internal rotation during horizontal landing in athletes with patellar tendon abnormality when compared with the normal control. The authors speculated that the altered kinematics on the hip and knee joints may increase the loading on the medial side

of the proximal patellar tendon during landing. The impaired strength of the abductors and external rotators of the hip has been identified as a major risk factor for the development of knee problems such as patellar femoral pain syndrome (Ireland et al. 2003; Cichanowski et al. 2007) and iliotibial band syndrome (Fredericson et al. 2000). To date, no studies have compared the hip strength in athletes with and without patellar tendinopathy. In view of the high prevalence of patellar tendinopathy among athletes participating in jumping sports, it is worth exploring whether hip strength is one of the risk factors for the development of patellar tendinopathy.

Lower muscle flexibility was found to increase the magnitude of loading on the patellar tendon during knee motion, and contribute to patella tendon overload (Fyfe et al. 1994). In a two-year prospective study, Witvrouw et al. (2001) found that the lower flexibility of the quadriceps and hamstrings contributed to the development of PT. Van der Worp et al. (2011) also proposed that reduced flexibility of the quadriceps muscles is a major risk factor for the development of patellar tendinopathy. They quantified muscle flexibility by measuring the range of knee motion using a plaster goniometer. The range of knee motion relies on the flexibility of the quadriceps muscles, the elasticity of the patellar tendon, and the mobility of the patella-femoral joint. To identify where (the muscle, tendon, or joint) and which components of the quadriceps muscles require targeted intervention, direct measurement of the muscle stiffness is warranted.

The aims of this study were to compare the hip muscle strength (abductors and external rotators) and the stiffness of the patellar tendon and thigh muscles in jumping athletes with and without PT and to explore the associations between hip muscle strength,

the elasticity of the patellar tendon, and the VL and RF muscles. We hypothesized that athletes with PT would have weaker muscle strength in the hip abductors and external rotators and increase muscle stiffness in the VL and RF muscles than healthy controls. Additionally, weaker strength in the hip abductors and external rotators; greater stiffness in the VL and RF muscles would associate with greater stiffness of the patellar tendon.

4.3 Material and methods

4.3.1 Subject recruitment

Male athletes with PT were recruited from local volleyball and basketball teams whom had fulfilled the following criteria. Subjects were 1) between 18 and 35 years of age; had 2) pain in the inferior pole of patella or the proximal part of patellar tendon aggravation during single leg squatting and jumping (Lian et al. 1996); 3) pain duration >3 months; 4) maximum intensity of pain in the previous week >3 using a visual analogue scale (VAS) with 0 as no pain and 10 as the worst pain; 5) VISA score <80 (Zwerver et al. 2011); 6) no history of corticosteroid injection and surgery to the lower limb; and 7) thickening of proximal part of patellar tendon with area of hypoechoic signals (Kulig et al. 2013). Age-matched subjects from the same teams with no past history of knee trauma or surgery and not having anterior knee pain or inflammation were invited as control. The invited subjects would be physically assessed by an experienced physical therapist having 13 years of clinical experience and then received ultrasonography examination from another physical therapist with 3 years of performing ultrasonography. For subjects with bilateral PT, measurements were taken on the more painful leg (Bolgla et al. 2008).

4.3.2 Measurements on muscle strength

Isometric muscle strength of the hip abductors

The isometric strength of the hip abductors was tested using a Nicholas hand-held dynamometer (Lafayette Instruments, Lafayette, IN, U.S.A). The subjects were tested in a side-lying position with the examined leg placed uppermost and parallel to the examination table (Fig. 4.1A). The center of the force pad of a hand-held dynamometer was placed over a mark located 5 cm proximal to the lateral knee joint line. The subject was instructed to push the leg upward with maximal effort and to hold for 5 seconds. During testing hip and knee joint maintained neutral position. After one trial, three measurements were made, with a 15-second rest between each trial. The mean value from the three measurements was used for further analysis (Fredericson et al. 2000; Ireland et al. 2003). This procedure has been shown to have high test-retest reliability of (ICC=0.97) (Fredericson et al. 2000).

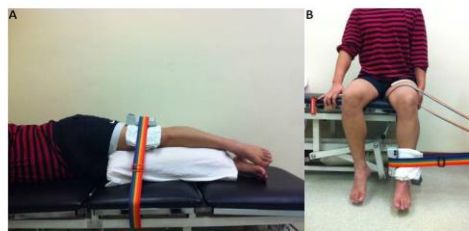


Figure 4.1 The hand-held dynamometer was used to measure the isometric strength of (A) hip abductor and (B).external rotators

Isometric muscle strength of the hip external rotators

The strength of the hip external rotators was also measured by the Nicholas hand-held dynamometer. The subjects were tested while sitting with the trunk kept upright and the hips and knees flexed to 90° of knee flexion (Fig.4.1B). The tested leg was stabilized by a strap in order to avoid the hip adduction. The center of the force pad of the dynamometer was placed 5 cm above the medial malleolus. The subject was instructed to push the leg inward with maximal effort and hold for 5 seconds. Three measurements were taken after a practice trial, with a 15 second rest between trials. The mean value of the three measurements was normalized with body weight for further analysis (Fredericson et al. 2000; Ireland et al. 2003).

4.3.3 Measurement on tendon and muscle elastic properties

Supersonic shearwave imaging (SSI) was conducted using an Aixplorer® ultrasound unit (Supersonic Imaging, Aix-en-Provence, France) in conjunction with a 50 mm linear-array transducer at 4-15 MHz frequency to measure the tendon and muscle shear elastic modulus. The musculoskeletal acquisition mode was used with the temporal averaging (persistence) and spatial smoothing set to medium and six, respectively. The elastic images were taken at 1 Hz.

Measurement on the elastic properties of the patellar tendon

Similar procedure was conducted as delineated in chapter 2. In brief, participant was examined in supine lying with 30° of knee flexion. B-mode was used to locate and align the patellar tendon longitudinally with the transducer. When a clear image of the patellar tendon was captured, the shear wave elastography mode was then activated. The transducer was stationed on the skin with light pressure on top of a generous amount of

coupling gel, perpendicularly on the surface of the skin. The transducer was kept stationary for 8-12 seconds during the acquisition of the shearwave elastography map (Kot et al. 2012). A total of 3 images were captured for the tendon on each knee for off-line analysis.

Off-line analysis was conducted using the procedures described in a recent paper (Kot et al. 2012). In brief, the region of interest (ROI) was first defined by a rectangular box of 13.5 mm×12.5 mm (biggest size provided by the manufacturer) distal to the apex of the patella and with the patellar tendon located in the center. In the painful tendon, the circular quantification box (Q-BoxTM) was centered where hypoechogenicity, disruption, or fragmentation of the collagen fiber, or focal sonolucency were observed. The diameter of the Q-Box was determined by the width of the tendon. In the non-painful tendon, the Q-BoxTM was centered on the proximal part of the patellar tendon with consistent images, and about 5 mm from the apex of the patella.

Measurement of the elastic properties of vastus lateralis and rectus femoris muscles

The quadriceps muscles of interest were the vastus lateralis muscle and the rectus femoris muscle. The scanning sites for the muscles were determined as follows: the vastus lateralis: distal 2/3 along the line from the anterior superior iliac spine (ASIS) to the lateral side of patella; and the rectus femoris muscle: mid-point from the ASIS to the superior part of patella. These sites were selected with reference to the recommended placement of the surface electrodes for electromyography (EMG) (Hermens 1999). The sites were located and marked with an eye-liner pencil. The muscles were first identified using the conventional grey-scale of the ultrasound unit. Once the muscles were identified, the probe was aligned with the fibers of the testing muscles (Eby et al. 2013).

The shearwave elastography mode was activated to measure the muscle shear elastic modulus. The probe was stationed on the skin with light pressure for 8-12 seconds (Kot et al. 2012) (Fig. 4.2). The images were taken when the color in the region of interest was uniform and stored for off-line analyses. Three images were captured for each muscle. A circle delineating the ROI was centered on the tested muscle. The diameter of the ROI was defined by the thickness of the muscle, which was determined by the distance between the superficial and deep muscle fasciae (Nordez and Hug. 2010).

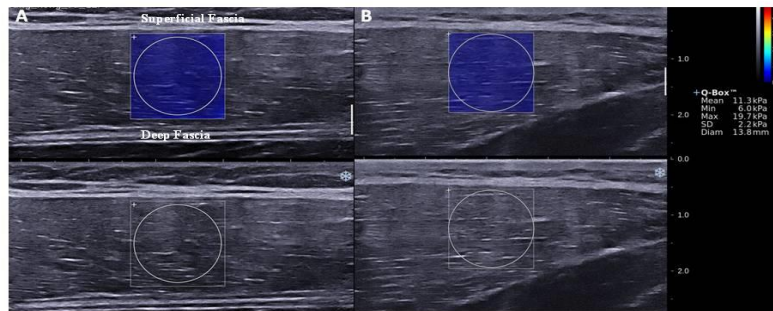


Figure 4.2 Upper images show color-coded box presentations of (A) RF and (B) VL muscle on elastograph superimposed on a longitudinal grey scale sonogram of RF and VL muscles, with the circle representing the region of interest and its corresponding elastic modulus demonstrating under Q-BoxTM on the right. Bottom images show longitudinal grey scale sonograms of RF and VL muscles on the identical scan planes.

4.3.4 Data reduction

The mean values of the Young's modulus within the Q-BoxTM were computed and displayed in kPa on the computer screen. The mean tendon and muscle shear elastic modulus were calculated by dividing the Young's modulus generated from the system by three (Royer et al. 2011). The shear elastic modulus of the patellar tendon has been

shown to have excellent test-retest reliability (with ICC=0.98) (Zhang and Fu 2013). The ICCs of the shear elastic modulus of the VL and RF muscles was 0.89 and 0.80, respectively (unpublished data on 30 subjects).

4.3.5 Statistical analysis

SPSS version 17.0 (SPSS Inc, Chicago, IL) was used to perform the statistical analyses. The data normality of the variables was assessed using the Shapiro-Wilk test. The continuous variables were expressed as the mean (standard deviation). Chi-square tests were used to examine the difference in sports between the PT group and the Control group. Independent *t*-tests were performed to compare the demographic data between players with and without PT. Paired *t*-tests were used for side-to-side comparisons of the variables in the healthy controls. If no significant side-to-side differences in the variables in the healthy controls, multivariate analysis of covariance (MANCOVA) tests were used to compare the hip muscle strength (of the abductors and external rotators) and the shear elastic modulus of the quadriceps muscles (VL and RF) muscles and patellar tendon between the painful side in athletes with PT and the dominant side of the controls with variables with significant group difference as covariates. Receiver operating characteristic (ROC) curve analyses were conducted on the variables with significant group differences. Youden's index was computed to define the threshold value to differentiate athletes with and without patellar tendinopathy. The area under the curve (AUC) was also reported. Pearson correlations were used to examine the relationships between the shear elastic modulus of the patellar tendon, the hip strength (abductor and external rotator), and the shear elastic moduli of the thigh muscles (VL and RF) in the

athletes with and without PT. Linear regression analysis was performed on the related variables. The P values < 0.05 were considered to be statistically significant.

4.4 Results

4.4.1 Subjects characteristics

The age, height, weight, BMI, and training intensity of the participants in the two groups are shown in Table 4.1. There were no significant differences in age, height, and weight and sports ($p>0.05$), but significant differences were found in BMI ($p=0.032$) and training hours per week between the two groups ($p=0.011$) (Table4.1). BMI and training hours were used as covariates for further analysis.

Table 4.1 Demographic data between PT and control group

Variables	PT Group (n=36)	Control Group (n=30)	p
Age (y)	22.8±4.2	23.5±4.6	0.504
Weight (kg)	74.1±6.6	72.5±8.4	0.357
Height (cm)	180.1±5.9	182.0±5.9	0.203
BMI (kg/m ²)	22.9±1.9	21.8±2.0	0.032
Sport-specific training (h/wk)	6.3±3.5	8.7±4.0	0.011
Sports (volleyball/basketball)	17/19	15/15	0.509
Pain duration (y)	2.6±1.7		
Unilateral/Bilateral PT	18/18		

Values shown as mean± standard deviation; PT:patellar tendinopathy; BMI: body mass index.

4.4.2 Side-to-side comparisons in the healthy controls

No significant differences were found in the strength of the hip abductors and external rotators in the dominant and non-dominant sides of the healthy controls ($p>0.05$) (Table 4.2). In addition, there were no significant side-to-side difference in the shear elastic modulus of the VL and RF muscles or the patellar tendons ($p>0.05$) of the healthy controls (Table 4. 2).

Table 4.2 Side-to-side comparisons of the hip muscle strength and shear elastic modulus of muscles and patellar tendon in the healthy subjects

Variables	Dominant Side	Non-dominant Side	<i>p</i>
Normalized muscle strength (%BW)			
hip abductors	42.9±5.8	42.4±5.9	0.409
hip external rotators	18.8±3.1	18.9±1.9	0.858
Shear elastic modulus (kPa)			
VL muscle	3.6±0.5	3.6±0.5	0.808
RF muscle	3.9±0.9	4.0±0.8	0.650
Patellar tendon	27.1±8.7	27.3±11.2	0.922

Values shown as mean±standard deviation; VL= vastus lateralis; RF=rectus femoris

4.4.3 Comparisons between athletes with and without PT

The athletes with PT exhibited significantly lower strength in the hip abductors (by 18.2%; $p=0.000$) and external rotators (by 11.2%; $p=0.007$) compared with the controls (Table 4.3; Fig.4.3). Furthermore, the athletes exhibited higher shear elastic modulus of the VL muscle (by 26.5%; $p=0.000$) and patellar tendon (by 48.0%; $p=0.000$) compared with the controls (Table 4.3).

Table 4.3 Comparisons of the normalized hip strength and elastic modulus of thigh muscles and patellar tendon on the painful side of players with PT and dominant side of the healthy players

Variables	PT Group (n=36)	Control Group (n=30)	<i>p</i>
Normalized muscle strength (%BW)			
hip abductors	35.1±6.0	42.9±5.8	0.000
hip external rotators strength	16.7±3.0	18.8±3.1	0.007
VL shear elastic modulus (kPa)	4.9±0.9	3.6±0.5	0.000
RF shear elastic modulus (kPa)	3.9±0.6	3.9±0.9	0.831
Patellar tendon shear elastic modulus (kPa)	52.1±20.9	27.1±8.7	0.000

Values are mean±SD . VL:vastus lateralis; RF: rectus femoris.

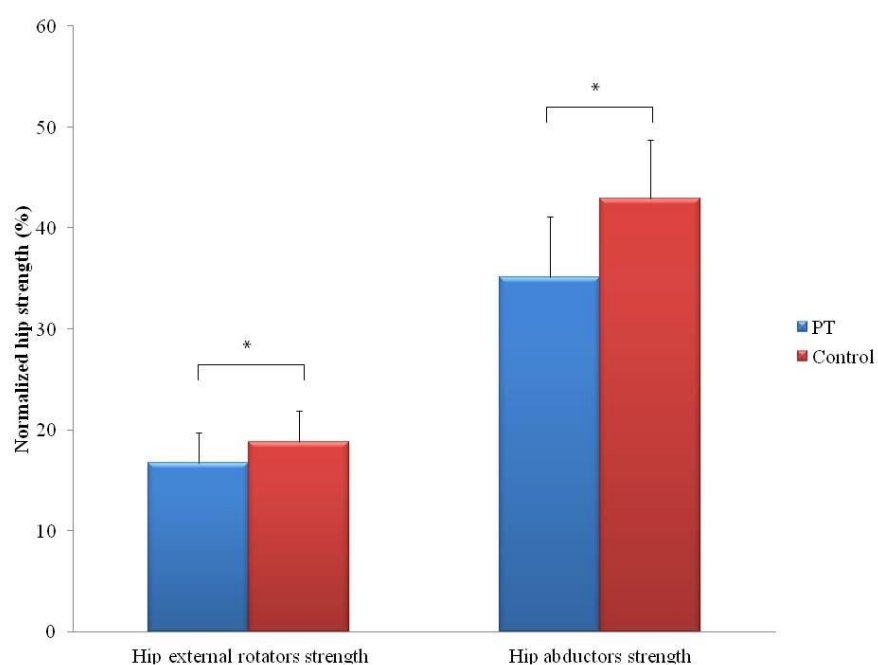


Figure 4.3 Comparisons in normalized muscle strength of the hip abductors and external rotators between painful side among players with PT and dominate side of control group. Significant difference in hip strength was found between 2 groups. *Players with patellar tendinopathy is significantly weaker than controls

Table 4.4 shows that the shear elastic modulus of the tendon patellar was significantly correlated with the normalized hip abductors strength ($r=-0.49$; $p=0.004$), normalized hip external rotators strength ($r=-0.4$; $p=0.025$) and the shear elastic modulus of the VL muscle ($r=0.38$; $p=0.035$) in the volleyball players. In the basketball players, the shear elastic modulus of the patellar tendon was significantly correlated with normalized hip abductors strength ($r=-0.55$; $p=0.001$) and the VL shear elastic modulus ($r=0.41$; $p=0.016$). Hence, the higher shear elastic modulus of the patellar tendon was associated with weaker hip muscle strength and a higher shear elastic modulus of the VL.

Table 4.4 Correlations between patellar tendon elastic modulus with normalized hip muscle strength as well as shear elastic modulus of thigh muscles of the affected leg.

Variables	<i>r</i>	<i>p</i>
Volleyball players		
Normalized hip abductors strength	-0.49	0.004
Normalized external rotators strength	-0.40	0.025
Total hip strength	-0.53	0.002
VL shear elastic modulus	0.38	0.035
RF shear elastic modulus	-0.12	0.503
Basketball players		
Normalized hip abductors strength	-0.55	0.001
Normalized external rotators strength	-0.27	0.151
Total hip strength	-0.53	0.002
VL shear elastic modulus	0.41	0.016
RF shear elastic modulus	0.07	0.698
Volleyball+basketball players		
Normalized hip abductor strength	-0.51	0.000
Normalized external rotator strength	-0.34	0.005
Total hip strength	-0.53	0.000
VL shear elastic modulus	0.38	0.001
RF shear elastic modulus	-0.05	0.699

VL: vastus lateralis; RF: rectus femoris; Hip strength: a percentage of body weight.

ROC curves were constructed to determine the optimal cutoff point of shear elastic modulus of the patellar tendon and VL muscle as well as the hip muscle strength

in differentiating players with and without PT. The Youden's indexes of the normalized muscle strength of the hip abductors was 35.7% with a sensitivity of 65.7% and specificity of 90.0% and of the hip external rotators was 17.9% with a sensitivity of 68.6% and specificity of 70.0% (all $p < 0.05$; Table 4.5; Fig. 4.4A; Fig. 4.4B). Patellar tendon with shear elastic modulus had Youden's index of 39.4 kPa with a sensitivity of 75.0% and specificity of 93.3% ($p < 0.05$; Table 4.5; Fig. 4.4C). The Youden's index of the VL shear elastic modulus was 4.2 kPa with a sensitivity of 86.1% and a specificity of 90.0% ($p < 0.05$; Table 4.5; Fig. 4.4D).

Table 4.5 Area under the curve, cutoff point value, sensitivity and specificity

Variables	AUC	Cut-off point	Sensitivity	Specificity
Normalized hip abductors strength (%)	0.84(0.000)	35.7%	65.7%	90.0%
Normalized hip external rotators strength (%)	0.71(0.004)	17.9%	68.6%	70.0%
Patellar tendon shear elastic modulus (kPa)	0.87(0.000)	39.4	75.0%	93.3%
VL muscle shear elastic modulus (kPa)	0.92(0.000)	4.2	86.1%	90.0%

AUC: Area under the curve; VL: vastus lateralis; hip strength: a percentage of body weight (kg)

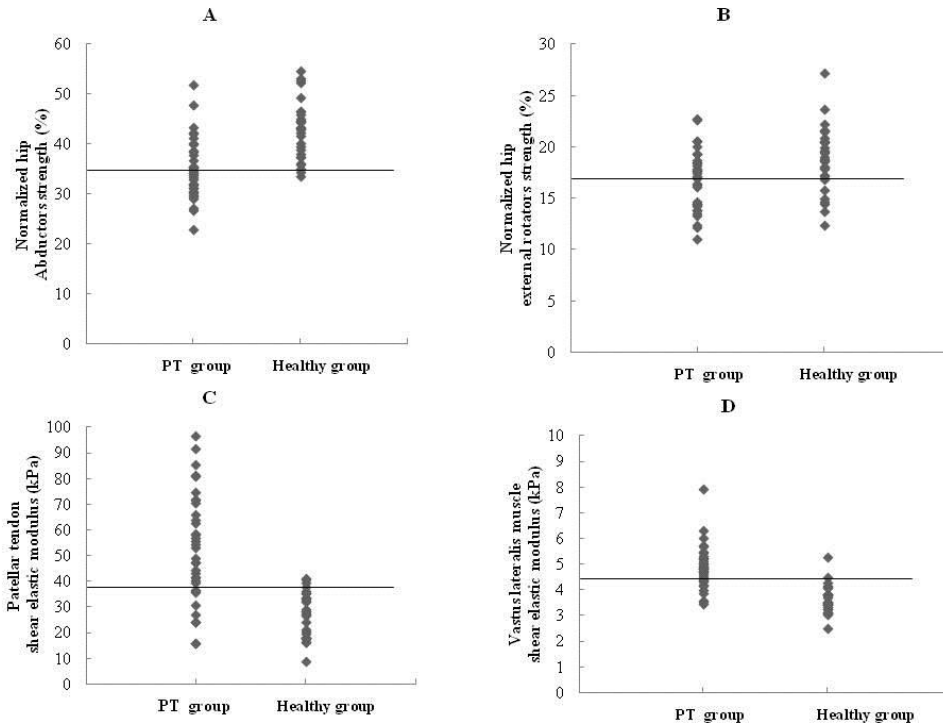


Figure 4.4 Scatter plots for (A) Normalized hip abductors strength (%),(B) normalized hip external rotators strength, (C) patellar tendon shear elastic modulus and (D) VL muscle shear elastic modulus in the healthy and players with PT showing the cut-off points. PT denotes patellar tendinopathy.

4.5 Discussion

The findings from this study indicated that the muscle strength of the hip abductors and external rotators of the athletes with PT were lower compared with the healthy controls. The athletes with PT had stiffer patellar tendons and VL muscles. The stiffness of the patellar tendon was associated with the stiffness of the VL muscle and hip muscle strength. In addition, the hip muscle strength and tendon and muscle stiffness could be used to identify athletes with and without PT.

Lower in hip muscle strength among players with PT

A significant lower in the muscle strength of the hip abductors and external rotators was detected in the basketball and volleyball players with PT when compared with healthy controls. The percentage of reduction in muscle strength was slightly higher in the hip abductors (by 18.2%) than the external rotators (by 11.2%). These findings were similar to those of Silva et al (2014), who found that the hip extensors of basketball and volleyball players with PT were significantly weaker (by 23.5%) than those of the healthy controls. The muscle strength of the hip extensor was also assessed using a handheld dynamometer. Hence, weakness in the major hip muscles was found in patients with PT on the hip abductors and external rotators in addition to the hip extensors. The optimal cutoff values for the muscle strength of the hip abductors (of 35.7%) and external rotators (of 17.9%) of basketball and volleyball players with and without PT were identified. The findings indicated that 90% of the players with normalized muscle strength of the hip abductors higher than 35.7% and 70.0% of the players with normalized muscle strength of the external rotators higher than 17.9% were free of tendon tendinopathy. These findings suggested that the muscle strength of the hip abductors played a more specific role in identifying players without tendinopathy than the hip external rotators. The sensitivity in identifying players with PT was fair (65.7%-68.6%). Together, these findings indicate that strengthening and monitoring the hip muscles, particularly the hip abductors, is important for the prevention of PT.

The hip abductors and external rotators may be important determinants of the kinematics of the lower limbs. Weakness in these muscles may induce the abnormal hip adduction and internal rotation during landing from jumps observed by Edward et al. (2010). The authors found greater hip adduction/internal rotation during landing in

athletes with patellar tendon abnormalities compared with athletes with normal patellar tendons. These altered landing patterns may cause excessive tensile stress on the medial side of the patellar tendon and lead to patellar tendon injury. In fact, most of the athletes with PT in this study experienced pain over the medial and central parts of the patellar tendon. Yu et al. (1995) also observed that tendinopathy occurred in the medial side of the proximal part of the patellar tendon. Fietzer et al. (2012) reported a 36% greater peak vertical ground reaction force in dancers with PT compared with the control group. Bisseling et al. (2007) also found that volleyball players with PT had a stiffer landing technique (greater knee extensor moment/lesser joint range of motion) compared to the control group. All of these changes may be associated with the impaired hip muscle that contributes to the abnormal landing kinematics and overloading on the patellar tendon.

Stiffer patellar tendon and VL muscle in athletes with PT

The patellar tendon and VL muscle of the volleyball and basketball players with PT were found to be stiffer compared with the control group. Sconfienza et al. (2010) reported that individuals with Achilles tendon pain had stiffer tendons in the tendon body than the healthy controls. However, this stiffness was not detected at the myotendinous junction or the calcaneal entheses. The measurements in this study were centered at about 5 mm from the apex of the patellar tip and were mainly taken in the tendon body, which explains why the findings are similar to those of Sconfienza et al. (2010).

In this connection, Witvrouw et al. (2001) concluded from a two-year prospective study that the lower flexibility of the quadriceps may contribute to the development of patellar tendinitis. In this study, SSI technology was used to directly measure the muscle

stiffness of the individual quadriceps components. Increased muscle stiffness was detected in the VL muscle of the subjects with PT but not in the RF muscle. It is unclear whether the landing loading differs between the two muscles. In fact, although the VL and RF muscles have similar types of muscle fibers, the VL muscle is monoarticular and RF is biarticular.

In this study, an optimal cutoff of the shear elastic moduli for the patellar tendon (39.4kPa) and the VL muscle (4.2kPa) were also delineated to identify players with and without PT. Both the tendon and the VL muscle elasticity had high sensitivity (over 75%) and specificity (over 90%) in identifying players with and without tendinopathy. These values were higher than those for the hip muscle strength. Hence, players with PT were able to be identified based on their tendon and muscle elasticity. However, the equipment is expensive and may not be available, the measurement can be time consuming, and few clinicians know how to conduct SSI. However, hip muscle strength can be evaluated in normal clinical settings and sports venue for quick screening, whereas SSI is recommended when accurate judgment is needed.

Associations between hip muscle strength and patellar and muscle elasticity

The weaker hip abductor/external rotator muscle strength in the basketball and volleyball players was found to be associated with a stiffer patellar tendon. These findings indicate the importance of hip muscle strength in preventing the reduction in tendon elasticity. The muscle-tendon units of the hip, knee, and ankle joints act to dissipate the kinetic energy on landing (Fredberg et al. 1999). The hip joint absorbs about 30% of the ground reaction force on landing (Decker et al. 2003). Weakness of the hip muscles may reduce the shock absorption and increase the vertical ground reaction force

loaded on the patellar tendon on landing from a jump. Indeed, a greater vertical ground reaction force was found in dancers with PT than in the healthy controls (Fietzer et al. 2012). Bisseling et al. (2008) also demonstrated that volleyball players with PT had a higher loading rate on the knee extensor moment during landing compared with the healthy controls. Together with altered landing kinematics and reduced hip muscle strength may induce excessive loading on the patellar tendon leading to an increase the stiffness of patellar tendon. Further assessments of the hip muscle strength, landing kinematics, and impact force would shed more light on the relationship between hip muscle strength and the landing kinematics and kinetics.

A positive relationship was also found between the patellar tendon and VL muscle stiffness. Note that the VL muscle is attached to the base and superolateral border of the patella and is connected to the lateral side of the patellar tendon via the lateral retinaculum (Becker et al. 2010). This anatomic relationship shows that the patellar tendon and VL muscle are closely related. Accordingly, tension in the VL muscle will induce tension in the patellar tendon. In a cadaveric study, Powers et al. (2006) found that the tension in the patellar tendon was affected by tension in the lateral retinaculum. The findings from this study further demonstrate the close relationship between the VL muscle and patellar tendon tension.

4.6 Limitations

Although weakness in the hip muscles was detected in players with PT, the cause-effect relationship could not be determined. Impaired hip muscle strength may lead to the development of PT. The presence of PT and its associated pain may reduce the loading on the affected limb and thereby induce disuse weakness in the hip muscles. A

prospective study is required to ascertain the cause and effect relationship between hip muscle strength and tendon stiffness. Second, only male subjects were recruited in this study because PT has higher prevalence in male athletes. Therefore, the generalizability of the findings is limited to male volleyball and basketball players. Finally, only the subjects with patellar tendinopathy were recruited in the present study. The findings cannot, therefore, be generalized automatically to other tendinopathies such as Achilles tendinopathy and supraspinatus tendinopathy. In view of anatomy, there are different anatomic features between patellar tendon (bone-to-bone) and Achilles tendon and supraspinatus tendon (bone-to-muscle). Further studies will be conducted to investigate the changes in elastic properties of Achilles tendon and supraspinatus tendon due to pathology.

4.7 Clinical implications

Lower the strength of hip abductors and external rotators is related to PT in male volleyball and basketball players. Strengthening programs for the hip abductors and external rotators is recommended for volleyball and basketball players to prevent PT. Stiffer patellar tendons and VL muscles were found in players with PT than in those without. In addition to localized treatment, treatment should include releasing the VL muscle stiffness and training the hip muscles of patients with PT. This information can aid physical therapist to educate the athletes with patellar tendinopathy to stretch quadriceps muscles and strengthen hip muscles (abductors and external rotators) as a home exercise.

4.8 Conclusions

Athletes with PT have less muscle strength in the hip abductor and external rotator muscles. Weakness in the hip muscles is associated with stiffness in the patellar tendon. These findings suggest that strengthening programs targeting the hip abductors and external rotators is recommended for the prevention of PT in volleyball and basketball players.

CHAPTER 5

CHANGES IN MORPHOLOGICAL AND ELASTIC PROPERTIES OF PATELLAR TENDON IN ATHLETES WITH UNILATERAL PATELLAR TENDINOPATHY AND THEIR RELATIONSHIPS WITH PAIN AND FUNCTIONAL DISABILITY

5.1 Abstract

Objectives: To compare the morphology and elastic properties of patellar tendons between athlete with and without unilateral PT and to examine its association with self-perceived pain and dysfunction.

Methods: In this cross-sectional study, 33 male athletes (20 healthy and 13 with unilateral PT) were enrolled. The morphology and elastic properties of the patellar tendon were assessed by the grey and elastography mode of supersonic shear imaging (SSI) technique while the intensity of pressure pain, self-perceived pain and dysfunction were quantified with a 10-lb force to the most painful site and the Victorian Institute of Sport Assessment-patella (VISA-P) questionnaire, respectively. Paired *t*-tests were used to compare the outcome measures (thickness, CSA and shear elastic modulus of patellar tendon) between the dominant and non-dominant sides in the healthy athletes, and also the painful and non-painful sides in athletes with unilateral PT. Univariate analysis of covariance tests were used to compare between the affected side in athletes with PT and the dominant side of the controls with demographic factors that demonstrated significant group difference as covariates. Spearman's rank correlation tests were used to examine the thickness ratio, CSA ratio and elastic ratio with the pressure pain, individual and total scores of the VISA-P questionnaire.

Results: In athletes with unilateral PT, the painful tendons had higher shear elastic modulus and larger tendon than the non-painful side ($p < 0.05$) or the dominant side of the healthy athletes ($p < 0.05$). Significant correlations were found between tendon shear elastic modulus ratio (shear elastic modulus of painful over non-painful tendon) and the intensity of pressure pain ($\rho = 0.62$; $p = 0.024$), VISA-P scores ($\rho = -0.61$; $p = 0.026$),

and the sub-scores of the VISA-P scores on going down stairs, lunge, single leg hopping and squatting (*rho* ranged from -0.63 to -0.67; $p < 0.05$).

Conclusions: Athletes with unilateral PT had stiffer and larger tendon on the painful side than the non-painful side and the dominant side of healthy athletes. No significant differences on the patellar tendon morphology and elastic properties were detected between the dominant and non-dominant knees of the healthy control. The ratio of the shear elastic modulus of painful to non-painful sides was associated with pain and dysfunction among athletes with unilateral PT.

Keywords: patellar tendinopathy; elastic properties; supersonic shear imaging; pain; VISA-P

5.2 Introduction

Patellar tendinopathy (PT) is a common and often chronic knee disorder among competitive athletes (Witvrouw et al. 2001). Its prevalence has been reported to be as high as 30% to 45% in athletes involved in jumping sports (Lian et al. 2005). Subjects with PT are characterized with localized pain at the proximal patellar tendon associated with jumping and squatting activities that load the tendon (Cook et al. 2000). Since the primary function of tendon is to transmit tensile loading, any change in its morphology and elastic properties may affect its function during normal activities.

Tendinopathy results in disruption and disorganization of the tendon fibers (Maffulli et al. 2004), along with increases in tendon thickness (Cook et al. 2000; Gisslen et al. 2005) and cross-sectional area (CSA) of the structure affected (Kuling et al. 2013). Based on ultrasound imaging, Cook et al. (1998) observed hypoechoic changes in human tendons with tendinopathy. The authors thereby recommended the use of ultrasonography in addition to clinical examination to confirm the diagnosis of PT. Alteration in the elastic properties of patellar tendon, however, have not been adequately described. In individuals with PT, the tendon was found to be more elastic in one study (Helland et al. 2013) but no difference was reported in another 2 studies (Couppé et al. 2013; Kongsgaard et al. 2010) when compared with controls. In those studies, tendon stiffness was assessed using ultrasound imaging with dynamometry. This technique measures the elastic properties of the whole tendon during ramped maximum voluntary isometric contraction. Clinically, pathological lesions in PT typically occur at about 5mm from the apex of the patella (Cook et al. 2000; Fredberg et al. 2008), therefore site-specific evaluation at the pathological region might shed light on the changes on tissue elastic

properties associated with tendinopathy.

Recently, strain imaging has been used to assess regional tendon elastic properties (De Zordo et al. 2009; Sconfienza et al. 2010). A compressive force is applied either manually or by the emission of low radiofrequency impulses via an ultrasound probe to the tendon surface causing tendon displacement. Tissue elasticity is graded as either soft, intermediate or hard and expressed in colour-coded images (elastogram) (Sconfienza et al. 2010). Based on this technique, the common extensor tendon was found softer in subjects with lateral epicondylitis (De Zordo et al. 2009) but harder among those with Achilles tendinopathy (Sconfienza et al. 2010) than healthy controls. To date, regional-specific evaluation on tendon elasticity associated with tendinopathy is scarce and findings are conflicting. In addition, the strain imaging technique provides qualitative but not quantitative information of tissue elasticity (Itoh et al. 2006; Klauser et al. 2010). Thus, the magnitude of changes could not be quantified.

The supersonic shear imaging (SSI) technique provides quantitative values of tendon elastic properties at a selected area of interest (Bercoff et al. 2004; Chen et al. 2013; Dewall et al. 2014; Kot et al. 2012; Zhang and Fu 2013). It relies on measuring the speed of propagation of shear waves generated by acoustic radiation force and to estimate the shear elastic modulus of soft tissues (Bercoff et al. 2004). Our recent findings indicated that the patellar tendon shear elastic modulus measured using SSI has good intra- and inter-rater reliability and is correlated with the Young's modulus of the tissue (Zhang and Fu 2013). Based on this technique, decreases in tendon elastic modulus in acute ruptured Achilles tendon in human subjects (Chen et al. 2013) and in partial tendon tears in a porcine model (Dewall et al. 2014) were reported. These studies provide the

evidence base on the feasibility of using SSI for measuring tendon elastic properties. However, these findings cannot be generalized to tendon with tendinopathy because this condition is a degenerative process (Khan et al. 1999). In addition to interruption of tendon fibrils (Maffulli et al. 2004), changes in fiber type (Ireland et al. 2001) and increases in collagen cross-link concentration (Kongsgaard et al. 2009) were detected in tendon with tendinopathy. Based on the SSI technique, quantitative regional-specific tendon elasticity could be measured and compared between subjects with and without patellar tendinopathy. Such information could increase our understanding in regional changes on tissue elastic properties as well as the magnitude of changes. Based on this approach, we have reported increase in tendon elasticity in players with PT when compared with healthy controls. We would like to explore further whether there would be difference on tendon elasticity between the painful and non-painful knee in players with unilateral patellar tendinopathy.

There is also a question of how the changes in tendon morphological and/or elastic properties in individuals with PT relate to their perceived pain and dysfunctions. Increased tendon thickness has been reported to be associated with greater pain among athletes with PT (Malliaras et al. 2010). To date, the relationship between elastic properties of tendon and self-perceived pain in individuals with PT has not been investigated. In view that pain and decrease in functional strength in the tendons could mean an end to the athletic career of a sportsman, it is therefore important to find out how changes in tendon morphology and mechanical properties are related to the disability and dysfunctions in people with PT, so that appropriate remedial measures can be developed.

The objectives of this study were to 1) compare the elastic modulus of patellar tendon between dominant and non-dominant sides among healthy subjects; 2) compare tendon on the painful and non-painful side of the same subject and also with healthy control subjects and; 3) determine whether changes in tendon shear elastic modulus were related to pain and dysfunction. We hypothesized that athletes with PT would have thicker and stiffer patellar tendon when compared with the non-painful side and tendon morphology and elastic properties might related with intensity of pain and functional disabilities in athletes with unilateral PT.

5.3 Materials and methods

5.3.1 Study population

Subjects were recruited from the volleyball, basketball and handball teams of local universities and the community. Only males were recruited, because PT is more prevalent in male than female athletes (Lian et al. 2005). The inclusion criteria were as follows: 1) between 18 and 35 years of age; 2) had unilateral pain in the inferior pole of patella or the proximal part of patellar tendon; 3) pain duration >3 months; 4) maximum intensity of pain in the previous week >3 using a visual analogue scale (VAS) with 0 as no pain and 10 as the worst pain; 5) VISA score <80 (Zwerver et al. 2011); 6) no history of corticosteroid injection or surgery to the lower limb. All recruited subjects were physically assessed by an experienced physical therapist who has 13 years of clinical experience and then ultrasonography examination was conducted by another physical therapist who has 3 years of experience in ultrasound scanning. The subject was diagnosed as having PT based on the clinical examination and ultrasonography findings

that included the following: 1) local tenderness in the inferior pole of patella, or the proximal part of patellar tendon; 2) pain aggravation during single leg squatting and jumping (Lian et al. 1996); 3) thickening of proximal part of patellar tendon with area of hypoechoic signals (Kulig et al. 2013). Twenty healthy athletes, with similar age and training hours but without clinical symptoms or abnormal ultrasound-based images of the patellar tendon, were recruited as the controls for this study.

All subjects filled in a form recording their age, weight, height and training duration per week. Subjects with PT completed the Victorian Institute of Sport Assessment-patella (VISA-P) questionnaire. The leg of dominance was determined by asking the subject to kick a ball (Bjornaraa and Fabio 2011).

Ultrasound Examination

An Aixplorer® ultrasound unit (Supersonic Imaging, Aix-en-Provence, France) in conjunction with a 50-mm linear-array transducer at 4-15 MHz frequency was used in this study (Bercoff et al. 2004). B-mode was used to measure the tendon thickness and CSA. Shearwave mode was used to measure the shear elastic modulus of the patellar tendon at its proximal part. The musculoskeletal acquisition mode was used to measure the elastic modulus of patella tendon using similar setting as reported in chapter 2.

Each participant was examined in supine lying with 30° of knee flexion (Bensamoun et al. 2006). The knee was supported on a firm towel and a custom-made ankle stabilizer to keep the leg in neutral alignment on the coronal and transverse planes. Prior to testing, the subject was allowed to have 5 minutes of rest in a comfortable

position in order to unload the tension on the patellar tendon (De Zordo et al. 2009). The room temperature was controlled at 25°C.

5.3.2 Measurement of patellar tendon thickness and CSA

The thickness and CSA in the inferior pole of patella were measured by grey scale mode (B-mode) of the Aixplorer® ultrasound unit. The inferior pole of patella was identified by palpation of the examiner. The transducer was lightly located at the inferior pole of the patella and the transducer was placed longitudinally on the patellar tendon. B-mode was activated to capture the image of the patellar tendon and stored for off-line measurements. The transducer was then turned by 90° so that a transverse view of the proximal insertion of the patellar tendon could be captured and stored for off-line analysis. Three images were obtained for measuring the thickness and CSA (Nyland et al. 2006). Both knees were evaluated for all the subjects.

After obtaining the ultrasound images, off-line measurements were performed. The tendon thickness was measured using the distance measurement software in the ultrasound machine. Measurements were taken from the inferior pole of patella vertically to the superior border of the patella tendon (Fig. 5.1A) using a trackball. The CSA of the patellar tendon (Fig. 5.1B) was measured by the tracing measurement software which allows the examiner to trace the outer margin of the tendon through the trackball, and the tracing measurement software was used to calculate the total area traced. The mean from the 3 measurements were used for statistical analysis.

Eleven healthy sedentary subjects were assessed twice with one week apart for test-retest reliability of patellar tendon thickness and CSA measurements. The inter-rater

coefficient of correlation of tendon thickness and CSA were 0.94 (CV=18.75%) and 0.98 (CV= 32.30%), respectively.

5.3.3 Measurement of patellar tendon elastic modulus

B-mode was used to locate and align the patellar tendon longitudinally with the transducer. When a clear image of the patellar tendon was captured, the shear wave elastography mode was then activated. The transducer was stationed on the skin with light pressure on top of a generous amount of coupling gel, perpendicularly on the surface of the skin. The transducer was kept stationary for 8-12 seconds during the acquisition of the SSI map (Kot et al. 2012). A total of 3 images were captured for the tendon on each knee for off-line analysis.

Off-line analysis was conducted and the procedures have been described in our recent paper (Kot et al. 2012). The region of interest (ROI) was first defined by a rectangular box of 13.5mm×12.5mm (biggest size provided from the manufacturer) distal to the apex of the patella and with the patellar tendon located within its centre part. In the painful tendon, the circular quantification box (Q-BoxTM) was centered where hypoechogenicity, disruption or fragmentation of collagen fiber, or focal sonolucent were observed. Similar to a previous study (Fredberg et al. 2008), the pathological lesions in our subjects were detected an average of 4.6 mm (ranged from 3-7 mm) distal to the apex of the patella (Fig. 5.1C). The diameter of the Q-Box was determined by the width of the tendon. In the non-painful tendon, the Q-BoxTM was centered at the proximal part of the patellar tendon with consistent images and at about 5mm from the apex of the patella. Young's modulus (E) was estimated by the SSI system based on the following equation.

$E = 3\rho c^2$, where ρ is the density (constant and equal to 1000kg/m^3) and c is the velocity of the shear wave propagation based on the assumption that the tissue is isotropic. A higher Young's modulus indicates greater stiffness (Bercoff et al. 2004). The mean and maximum values of Young's modulus within the Q-BoxTM were computed and displayed in kPa at the right bottom corner of the computer screen. The mean tendon shear elastic modulus was calculated by dividing the Young's modulus generated from the system by 3 (Royer et al. 2011). The SSI has excellent test-retest reliability on patellar tendon shear elastic modulus (ICC: 0.98; coefficient of variation: 29.53%) (Zhang and Fu 2013).

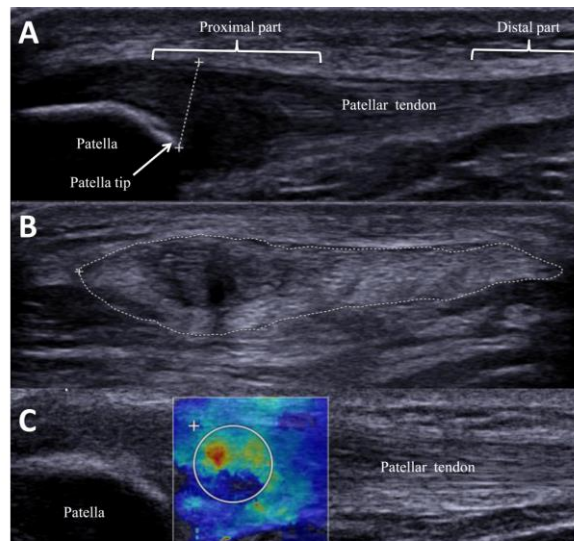


Figure 5.1 Sonography images of the patellar tendon (A) Thickness of the patellar tendon (dotted line) were measured from the superior border of the patellar tendon to the tip of the patellar. (B) Cross-sectional area of the patellar tendon was measured by tracing the outer margin of the patellar tendon (dotted circle) (C) Shear elastic modulus of the patellar tendon was quantified by the elastography. The white circle delineates the area of interest.

The ratio of mean shear elastic modulus, tendon thickness and CSA between the painful and non-painful knee was calculated as elastic ratio, thickness ratio and CSA ratio in athletes with unilateral PT.

5.3.4 Clinical evaluation

Pressure pain was measured by a hand-held algometer (manufactured by pdt, Rome, Italy). Pain was provoked through a rubber disc at the end of the algometer. The participant was positioned in supine lying with 30° of knee flexion on the couch. The most painful area on the proximal patellar tendon was determined by palpation and then a 10lb force was applied via the algometer onto this area (Fig. 5.2). The intensity of pain being provoked was reported using a visual analogue scale (VAS) from 0 to 10, with 0 indicating no pain, and 10 indicating the worst pain during testing. The VAS scale is a reliable and valid scale in the evaluation of patients with anterior knee pain (Crossley et al. 2004).



Figure 5. 2 Measurement of pressure pain on patellar tendon

The VISA-P questionnaire is used to assess the severity of symptoms and functional ability in subjects with PT (Visentini et al. 1998). The questionnaire comprises 8 questions with 4 on self-perceived pain associated with a functional activity, 2 on the ability in performing functional activities, and 2 on the ability to play sport. Self-perceived pain or abilities are rated on a 10-point Likert scale with 0 being the worst pain or lowest ability and 10 as the least pain and highest ability. The total score of the questionnaire is 100 and the final score would quantify the functional level. The VISA-P questionnaire has been used for studies on PT in various athletic populations (Frohm et al. 2004; Lian et al. 2005).

5.3.5 Statistical Analysis

Independent *t* tests were performed to compare the demographic data between athletes with and without PT. After the normality distributions were confirmed using the Shapiro-Wilk tests, paired *t*-tests were used to compare the outcome measures (thickness, CSA and shear elastic modulus of patellar tendon) between the dominant and non-dominant sides in the healthy athletes, and also the painful and non-painful sides in athletes with unilateral PT. Univariate analysis of covariance tests were used to compare between the affected side in athletes with PT and the dominant side of the controls with demographic factors that demonstrated significant group difference as covariates. Spearman's rank correlation tests were used to examine the thickness ratio, CSA ratio and elastic ratio with the pressure pain, individual and total scores of the VISA-P questionnaire. SPSS version 17.0 (SPSS Inc, Chicago, IL) was used to perform statistical analyses. A *p* value of <0.05 was considered as significant for each of the measurements.

5.4 Results

5.4.1 Participant's demographic data

Participants' age, height, weight, BMI and training intensity in the two groups are shown in Table 5.1. No significant differences were found between the two groups in age, height, weight, BMI and training intensity ($p>0.05$), but a medium trend was found in BMI between the 2 groups ($p=0.094$; Cohen's $d = 0.52$).

Table 5.1 Demography comparison between athletes with and without unilateral PT

Variables	Control group (n=20)	PT group (n=13)	<i>P</i> Value
Age (y)	24.9±4.4	22.9±4.6	0.222
Weight, (kg)	73.4±7.9	76.2±6.3	0.280
Height (cm)	181.7±6.0	180.0±5.7	0.425
BMI (kg/m ²)	22.2±2.1	23.6±2.4	0.094
Sport-specific training (h/wk)	7.5±3.7	5.4±2.5	0.102
Injury duration (y)		1.7±1.6	
Dominate/non-dominate side (painful side)		8/5	

Values shown as mean± standard deviation; PT=patellar tendinopathy; BMI=body mass index.

5.4.2 Side-to-side differences on thickness, CSA and shear elastic modulus

In athletes with unilateral PT, B-mode ultrasound measurements revealed significant differences between the painful and non-painful sides in patellar tendon thickness ($p=0.001$) and CSA ($p=0.002$) (Table 5.2). On average, the painful tendons were thickened by 33.3% and enlarged by 12.5% in CSA than the non-painful side. The

shear elastic modulus in the painful side (mean: 43.6 kPa) was significantly higher than the non-painful side (mean: 25.8 kPa) by 40.8 % ($p=0.008$).

Table 5.2 Comparisons of the shear elastic modulus, thickness and CSA between painful and non-painful sides in athletes with unilateral PT

Variables	Painful side (n=13)	Non-painful side (n=13)	<i>P</i> Value
Shear elastic modulus (kPa)	43.6±17.9	25.8±10.6	0.008*
Thickness (mm)	6.9±1.8	4.6±0.6	0.001*
CSA (cm ²)	1.7±0.4	1.4±0.3	0.002*

Values shown as mean± standard deviation; PT=patellar tendinopathy; CSA=cross sectional area. * $P<0.05$.

Side-to-side differences on the outcome measures were not observed in healthy athletes. There were no significant differences on the patellar tendon morphology and elastic properties between the dominant and non-dominant sides ($p>0.05$) despite a trend of increase in patellar tendon thickness in the dominant than the non-dominant leg ($p=0.095$) (Table 5.3). The mean tendon thickness, CSA and shear elastic modulus were 5.6 mm, 1.4 cm² and 27.5 kPa in the dominant leg; 5.3 mm, 1.4 cm², and 27.9 kPa in the non-dominant leg.

Table 5.3 Side-to-side comparisons of the shear elastic modulus, thickness and CSA in healthy athletes

Variables	Dominant side (n=20)	Non-dominant side (n=20)	<i>P</i> Value
Shear elastic modulus (kPa)	27.5±11.3	27.9±8.4	0.868
Thickness (mm)	5.6±1.2	5.3±1.0	0.095
CSA (cm ²)	1.4±0.3	1.4±0.3	0.917

Values shown as mean± standard deviation; CSA=cross sectional area.

5.4.3 Comparison of shear elastic modulus, thickness and CSA between healthy athletes and athletes with unilateral PT

Group differences were observed on the patellar tendon morphology and elastic properties between athletes with PT and without PT. The mean group differences on the patellar tendon thickness and CSA were 1.3 mm and 0.3 cm², respectively (Table 5.4). The shear elastic modulus was increased from 27.5 kPa to 43.6 kPa (by 36.9%, $p=0.003$) in the painful tendon among athletes with PT when compared with the controls.

Table 5.4 Comparisons of the shear elastic modulus, thickness and CSA of the patellar tendon on the painful side of athletes with unilateral PT and dominant side of the healthy athletes

Variables	Control group (n=20)	PT group (n=13)	<i>P</i> Value
Shear elastic modulus (kPa)	27.5±11.3	43.6±17.9	0.003*
Thickness (mm)	5.6±1.2	6.9±1.8	0.019*
CSA (cm ²)	1.4±0.3	1.7±0.4	0.032*

Values shown as mean± standard deviation; PT=patellar tendinopathy; CSA=cross sectional area. * $P<0.05$.

5.4.4 Relationships between changes in tendon morphology, elastic properties, pressure pain and dysfunctions

Table 5.5 shows the relationships between changes in tendon properties, pressure pain and dysfunctions. Significant negative correlation was found between elastic ratio and VISA-P scores ($\rho = -0.61$; $p=0.026$) (Fig. 5.3A). Significant positive correlation was found between elastic ratio and pressure pain ($\rho =0.62$, $p =0.024$) (Fig. 5.3B) and negative relationships were established between elastic ratio and self-perceived pain based on the sub-scores from the VISA-P questionnaire (ρ ranged from -0.63 to -0.67;

$p < 0.05$) (Fig. 5.3C, 3D, 3F, 3G, 3H) except for the knee extension ($\rho = -0.26$, $p = 0.394$) (Fig. 5.3E). A higher ratio, greater differences between the painful and non-painful tendon, was associated with greater intensity of pain with pressure and when performing forward lunge, going down stairs and single leg hopping; as well as greater dysfunctions. Similar relationships could not be detected with thickness ratio and CSA ratio.

Table 5.5 Spearman's rank correlations between the ratio of tendon thickness, CSA and shear elastic modulus of the painful and non-painful side with intensity of pressure pain, individuals and total VISA-P scores

Pain	Morphology		Elastic properties	
	Thickness ratio	CSA ratio	Elastic ratio	
Pressure pain	0.53	-0.04	0.62*	
Downstairs	-0.28	-0.22	-0.65*	
Knee extension	0.02	0.42	-0.26	
Single leg hopping	-0.30	-0.13	-0.64*	
Lunge	-0.11	-0.07	-0.67*	
Ability				
Prolong sitting	-0.46	-0.15	-0.63*	
Squatting	-0.14	0.00	-0.64*	
Dysfunction	VISA-p score	-0.25	-0.07	-0.61*

Abbreviations: CSA=cross sectional area; VISA-P=Victorian Institute of Sports Assessment-patella Questionnaire; * $p < 0.05$.

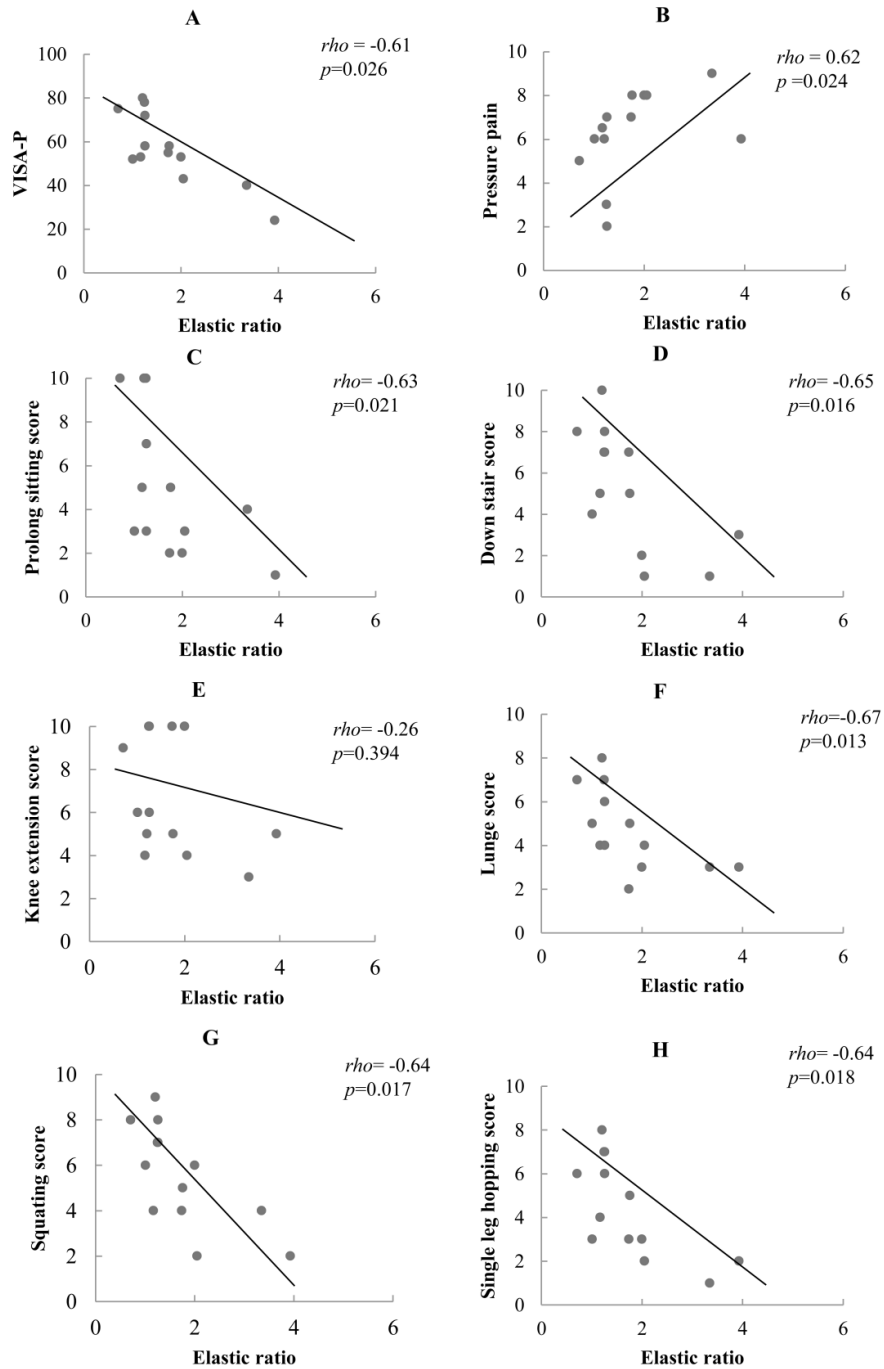


Figure 5.3 Correlations between elastic ratio and clinical variables. (Pressure pain, individual and total score from VISA-P, Victorian Institute of Sport Assessment

5.5 Discussion

The present study revealed changes in patellar tendon morphology and elastic properties in athletes with unilateral PT lasting from 3 months to 6 years. The painful tendons were thicker and larger in size with an increase in stiffness when compared with the non-painful side and healthy control subjects. This study also established associations between elastic properties of patellar tendon and intensity of pressure- and activity-related pain, as well as dysfunctions in basketball and volleyball players with unilateral PT.

Morphological changes such as thickening and larger CSA in pathological tendons, have been reported (Cook et al. 2000; Gisslen et al. 2005; Kuling et al. 2013) and the changes were about 22.7% in thickness (Gisslen et al. 2005) and 15.8% in CSA (Gisslen et al. 2005) in the proximal part of the patellar tendon. Cook et al. (Cook et al. 1998) even advocates the use of ultrasound imaging together with clinical examination in making diagnosis of tendinopathy. In this study, patients were confirmed to have PT based on both clinical examination and ultrasound imaging, it is therefore not surprising to find differences on the size and CSA of the pathological tendons from the unaffected side or the patellar tendon of the healthy controls. In the present study, the tendon size was increased by 35.7% and the CSA was enlarged by 21.4% in the pathological tendons. These changes could be explained by an increase in ground substance (Sharma and Maffulli 2005), collagen fiber disorganization (Maffulli et al. 2004), and hypercellularity (Kader et al. 2002).

One of the main findings from this study was the change in tendon elastic properties in athletes with unilateral PT using supersonic shear imaging technique. The

painful tendons were stiffer than the non-painful side as well as the dominant side of the healthy controls. The measurements were made on the proximal portion of the tendon where pain was elicited on palpation and perceived during functional activities of the lower limb. Patellar tendon elastic properties were previously quantified by ultrasonography with dynamometry. Kongsgaard et al. (2010) did not find any difference in the tendon stiffness between 9 healthy individuals and 8 subjects with patellar tendinopathy. A later study by Couppé et al. (2013) also reported no difference on the tendon stiffness between the painful and non-painful sides of 7 badminton players with unilateral PT and also compared with 9 control subjects. Based on a relatively larger sample, Helland et al. (2013) found significantly lower tendon stiffness (by 21.4%) in the patellar tendon in 13 volleyball players with PT when compared with 15 controls. In these studies, elastic properties of the whole tendon were measured so as to unveil the entire physical properties of the tendon. We were more interested to investigate regional-specific changes around the pathological region, where pain is normally elicited.

Similar to our study, regional-specific increase in tendon stiffness was reported in subjects with chronic pain in the Achilles tendons by Sconfienza et al (2010). The stiffness of Achilles tendons was assessed by ultrasound sonoelastography at the myotendinous junction, tendon body and calcaneal entheses. Loss of elasticity was detected in the tendon body but not in the myotendinous junction or calcaneal entheses when compared with control. On the contrary, De Zordo et al (2009) reported decrease in tendon stiffness at the common extensor origin in patients with lateral epicondylitis. More study is required to examine whether tendinopathy associated changes in elastic properties would be different in the lower and upper limb tendons.

The study from Sconfienza et al. (2010) observed lower elastic values in areas with fragmentation and loss of fibrillar texture. In our study, the tendon shear elastic modulus was measured at the proximal part of the patellar tendon in area of hypoechoic or fragmentation signals based on ultrasound imaging. On the contrary, lower tendon stiffness was detected in subjects with acute Achilles tendon rupture (Chen et al. 2013) and in partial tendon tears in a porcine model (Dewall et al. 2014). Chen et al. (2013) commented that the shearwave images could not be registered in areas with hematoma. The elastic value was dramatically reduced to 0 thus lowering the mean value. Furthermore, changes that occur in tendons with tendinopathy but not in tendons with acute rupture include transition of collagen fibers between Type I and Type III (Goncalves-Neto et al. 2002; Ireland et al. 2001); disorganization of collagen fibers (Maffulli et al. 2004), increase in collagen cross-links (Kongsgaard et al. 2009) and formation of scar tissues (Hooley and Cohen 1979). These changes would likely increase tissue elastic modulus. Biochemical and histological tests are suggested to assess changes on collagen fibers, extracellular matrix and tendon elastic modulus.

The patellar tendon is short and thick where considerable force is transmitted. An inextensible tendon would have the most efficient force transmission. This tendon also serves other important functions such as energy storage/release upon loading and unloading and protection from muscle fiber injury (Magnusson et al. 2003). To serve these functions, the patellar tendon exhibits spring-like characteristic, due to the presence of elastic components. Increased tendon stiffness in athletes with PT might be suitable for rapid and effective force transmission but could affect its function as mechanical buffer and elastic saving for economy of motion.

Besides measuring tendon elastic properties at rest, we also correlated the resting tendon elastic properties with pain and dysfunctions. Significant correlations were found between pain and dysfunctions with modulation on the tendon elastic properties but not with tendon morphology (thickness and CSA). Patients with PT are characterized with pain at the proximal patellar tendon associated with activities that load the tendon (Cook et al. 2000). The present findings provide evidence that a greater increase in stiffness of the painful tendon, i.e. greater ratio on the shear elastic modulus between the painful and non-painful side, are associated with the self-perceived pain with local pressure and during tendon-loading activities. The mechanism of tendon pain remains unclear, but previous studies suggested that the tendon pain may be related to neural pathways (nerve endings) (Danielson et al. 2006), neurotransmitters (glutamate and calcium ions) (Zhao et al. 1999) and ion channel (Magra et al. 2007). The correlation between pain intensity and tendon stiffness (the stiffer tendon; the greater pain intensity) may be associated with over-stretch the ion channel in tendon that is responsible for sensing and transmission nociception (Sachs 2010). In addition, based on “iceberg theory” the tendon pain may be attributed to be mechanical loading, adaption to mechanical loading and pain threshold (Abate et al. 2009).

Most importantly, the dysfunctions as reflected from the VISA-P scores are related with the modulation in the tendon elastic properties. Tendon pain is closely linked to loading while excessive energy storage and release (over stretch) to the tendon would most commonly provoke pain (Lichtwark and Wilson 2005). We could not establish a relationship between the change in tendon morphology with pain or dysfunction. Malliaras et al. (2010) found that hypoechoic area and diffuse thickening in patellar

tendon were more likely to be painful. However, Warden et al. (2007) and the present study could not establish a relationship between the CSA of patellar tendon and the VISA-P scores in athletes with unilateral PT.

Interestingly, there was not a significant side-to-side difference in the shear elastic modulus, thickness and CSA of patellar tendon between the dominant and non-dominant sides among healthy athletes. Such observations are partially in agreement with the findings from Couppé et al. (2008). The authors found similar shear elastic modulus between the dominant and non-dominant legs. The thickness and CSA of patellar tendon, however, were found to be significantly thicker and larger in the leading leg in fencers and badminton players when compared with the other leg. A proposed explanation for this finding is the unilateral/asymmetrical training in these athletes. In our study, we recruited athletes in volleyball, basketball and handball that are generally regarded as bilateral sports. .

5.6 Limitations

The prevalence of PT is reported to be between 30% and 45% in the jumping athletes (Lian et al. 2005), but many of them suffered from bilateral PT. During the study period, 13 subjects with unilateral PT were recruited. Despite this small sample size, statistical significant difference was established on patellar tendon shear elastic modulus and thickness between athletes with and without PT. We also detected correlations between the tendon elastic modulus and the intensity of pain and functional scores. Such findings illustrated influence of tendon stiffness on pain and function. However, further study with larger number of subjects and in female athletes are suggested to support the

present findings. Also, we conducted evaluation of tendon elastic properties on the proximal patellar tendon that does not represent the entire tendon. This is valid because the pathological changes in patellar tendon occurred mostly in the inferior pole of patella or proximal part of the tendon (Kulig et al. 2013). However, further study measuring tendon elastic modulus at different portions of the tendon would provide information on whether changes are isolated at the pathological region. In addition, ultrasound imaging was used to determine pathological lesions that had not been verified with histological tests. The present study was a cross-sectional study, we could not determine whether a stiffer proximal patellar tendon was the cause or consequence of the PT. Finally, only male athletes were recruited in this study, thus the findings from this study may not be generalized to female basketball, handball and volleyball players.

5.7 Conclusions

The present study revealed changes in both morphology and elastic properties at the painful part of patellar tendon in athletes with unilateral PT. The affected tendons are stiffer, thicker and have larger cross-sectional area than the non-painful side and the tendon of healthy controls. In addition, the ratio of the painful and non-painful tendon elastic properties is associated with the intensity of pressure pain and VISA-p scores in athletes with unilateral PT.

CHAPTER 6

**IMMEDIATE EFFECTS OF ONE SESSION OF
EXTRACORPOREAL SHOCK WAVE THERAPY IN ATHLETES
WITH PATELLAR TENDINOPATHY**

6.1 Abstract

Background: Patellar tendinopathy is one of the most common sport injuries in jumping athletes. Changes in the tendon mechanical properties have been detected in athletes with PT. Extracorporeal shockwave therapy (ESWT), a mechanical and pain provoking intervention, has been found to be effective in reducing the pain associated with PT. However, it is not known whether the reduction in pain induced by ESWT is associated with the modulation of the tendon elastic properties.

Objectives: To examine the immediate effects of ESWT on the elastic properties of the patellar tendon and the VL muscle and the RF muscle; and to explore the possible relationships between the changes in tendon resilience, muscle compliance, and the intensity of pain.

Study design: A single-blinded randomized controlled trial

Methods: Thirty-six male athletes aged between 18 and 32 with PT for more than 3 months (ranged from 3 to 72 months) were recruited from local basketball, volleyball, and baseball teams. The subjects were randomly assigned to the ESWT or sham groups. The subjects in the ESWT group received a session of ESWT at their maximum tolerable pain level at 4Hz for 1500 impulses. The minimum treatment intensity (less than 0.08 mJ/mm²) at 4Hz for 1500 impulses was applied to the subjects in the sham group. SSI was used to measure tendon and muscle stiffness. The muscles of interest were the VL and RF muscles. A hand-held algometer was used to apply 10 lb pressure on the tender spot of the affected tendon. The intensity of the pressure pain was quantified using the visual analogy scale (VAS). A single-legged declined-squat test (SLDST) was used to

assess the angle of knee flexion when pain was first perceived and the intensity of the first-perceived pain (based on the VAS). All of these outcome measures were conducted before and immediately after the application of ESWT. The percentage changes in the tendon and muscle shear elastic modulus, the intensity of pressure pain and squatting pain, and the composite of the knee range and intensity of pain were computed. Univariate analysis of variance tests were used to assess the between group differences on the outcome variables. Between-leg differences were assessed using univariate analysis of variance tests within the ESWT and sham groups. Pearson correlation coefficient tests were conducted to examine the relationships between the percentage changes in the tendon and muscle shear elastic modulus, pressure pain, SLDST_{pain}, and SLDST_{pain_angle} in the ESWT and sham groups.

Results: Significantly greater reduction in the tendon shear elastic modulus was detected in the ESWT group compared with the sham group ($p<0.05$). The patellar shear elastic modulus was significantly reduced by 24.7% and 8.0 % in the ESWT and sham groups, respectively. A significant reduction in the stiffness of the VL muscle was also observed in the ESWT group (by 18.8%) compared with the sham group (by 10.6%, $p=0.041$). In the ESWT group, the change in the tendon shear elastic modulus was related to the change in the intensity of squatting pain and the composite change in the knee range and squatting pain ($r= 0.52$ and 0.59 , respectively; all $p<0.05$). In the sham group, the change in the tendon shear elastic modulus was related to the change in the VL shear elastic modulus ($r=0.62$, $p=0.006$). No significant changes in the tendon and muscle shear elastic moduli were detected in the non-treated leg in the ESWT and sham groups.

Conclusions: When delivered at the maximum tolerable pain intensity, ESWT induced a significant reduction in tendon stiffness. The increase in tendon compliance was associated with pain reduction during the single-legged declined-squat test. Our findings further demonstrate the relationship tendon elasticity and pain in athletes with patellar tendinopathy. These findings suggest that the change in the tendon mechanical properties may be one of the mechanisms induced by ESWT in reducing the pain associated with patellar tendinopathy. Further research is needed to explore the long-term effects of ESWT on the mechanical properties of the patellar tendon and VL muscle, and their association with treatment efficacy.

Keywords: extracorporeal shockwave therapy, elastic properties of the patellar tendon, patellar tendinopathy, single-legged decline squat test

6.2 Introduction

Patellar tendinopathy (PT) is one of the most common injuries in jumping sports. Clinically, patients complain of pain localized at the proximal insertion of the patellar tendon, particularly during squatting and jump-land activities (Cook et al. 2000). Changes in tissue morphology (Cook et al. 2000; Gisslen et al. 2005; Kuling et al. 2013), vascularity (Richards et al. 2001; Giombini et al. 2013), and cellular activity (Leadbetter et al. 1992; Sharma et al. 2005) have been reported in the affected tendon. In a recent study, a decrease in tendon compliance was detected in patients with PT, and the increase in tendon stiffness was found to be related to the intensity of self-perceived pain (Zhang et al. 2014).

Extracorporeal shockwave therapy (ESWT) is a conservative intervention for treating individuals with PT. Extracorporeal shockwave are acoustic waves characterized by a high positive peak pressure and a rapid rise time (Ogden et al. 2001). In a recent review study, van Leeuwen et al. (2009) concluded that ESWT appears to be a safe and promising modality for reducing pain and improving function in individuals with PT. The conclusion was based on a review of seven studies. However, only three of the studies were randomized controlled studies. Taunton et al. (2003) and Wang et al (2007) reported a significant reduction in pain at three months after 1 to 5 sessions of ESWT at 0.17 to 0.18mJ/mm² when delivered 1500-2000 impulses. In contrast, when high intensity ESWT (0.25 to 0.42mJ/mm²) was prescribed at 4 Hz for 2000 impulses, no significant reduction in pain was detected compared with the control group. These findings suggest that medium intensity ESWT is effective in treating PT.

Despite its effectiveness in treating tendinopathy, the underlying mechanism of ESWT remains unclear (Wang et al. 2012; van der Worp et al. 2013). The proposed treatment mechanisms include increased collagen synthesis (Hsu et al. 2004) and growth factors (Chen et al. 2004; Caminoto et al. 2005), modulation of vascularization (Wang et al. 2002, Wang et al. 2003), and decreased nerve conduction (Hausdorf et al. 2008; Wu et al. 2008). Hyperstimulation analgesia is another proposed mechanism because pain is normally perceived during ESWT (Ogden et al. 2001; Notarnicola and Moretti 2012; Zwerver et al. 2011). In some studies, ESWT was prescribed at the patients' maximum tolerable pain level (Vetrano et al. 2013; Zwerver et al. 2010). However, it remains unknown whether ESWT can induce a change in pain sensitivity. Increased pain pressure sensitivity at the tibialis anterior tendon was observed when hypertonic saline was injected in the healthy tendon tissues (Gibson et al. 2006, Slater et al. 2011). This finding suggested that pain pressure sensitivity can be modulated with experimentally-induced pain.

In addition, experimentally induced pain can have motor and neural consequences. When hypertonic saline was injected into muscles, inhibition of the muscle activities in the tibialis anterior (Graven-Nielsen et al. 1997), the masseter (Sevensson et al. 1998), and the upper trapezius (Madeleine et al. 2006) was detected during maximal voluntary contraction. Pain induced by the injection of saline into the infrapatellar fat pad was also found to alter the delay in muscle onset of the vastus medialis and lead to a reduction in muscle activities in the vastus lateralis muscles during stepping activities (Hodges et al. 2009). These findings suggested that the alteration in muscle activities was induced by pain from a non-muscle origin, suggesting that the alterations in muscle activation may

be due to central rather than peripheral effects. Furthermore, modulation of the corticospinal excitability of muscles in the contralateral limb was reported in the hand (Kofler et al. 2001) and arm (Hoeger Bement et al. 2009) muscles with experimentally induced pain. If experimentally induced pain can modulate muscle activities, ESWT may induce a reduction in the muscle activities in the ipsilateral and contralateral limbs. This information would shed light on the therapeutic mechanisms of ESWT.

The aims of this study were to examine the immediate effects of ESWT on resting tendon and muscle stiffness, and to explore the possible relationships between changes in resting tendon resilience, muscle compliance, pressure pain, and squatting pain. In this study, one session of ESWT was hypothesized to lead to a reduction in tendon and muscle stiffness and the intensity of pressure pain. The changes in patellar tendon stiffness were found to be related to the reduction in pain during a single-legged decline-squat test.

6.3 Materials and methods

6.3.1 Subject recruitment

Thirty-six male subjects with PT were recruited from local university and community volleyball, basketball, and handball teams. Only males were recruited, because PT is more prevalent in male athletes (Lian et al. 2005). The inclusion criteria were as follows: 1) between 18 and 35 years of age (Zwerver et al. 2011); 2) pain in the inferior pole of the patella or the proximal part of the patellar tendon; 3) pain duration >3 months; 4) maximum intensity of pain in the previous week >3 using a visual analogue scale (VAS) with 0 as no pain and 10 as the worst pain; 5) VISA score <80 (Zwerver et al.

2011); and 6) no history of corticosteroid injection or surgery to the lower limb. All recruited subjects were physically assessed by an experienced physical therapist with 13 years of clinical experience. An ultrasonography examination was then conducted by another physical therapist with three years of experience in ultrasound scanning. The subjects were diagnosed as having PT based on the clinical examination and ultrasonography findings, which included: 1) local tenderness in the inferior pole of the patella, or the proximal part of the patellar tendon; 2) pain aggravation during single-leg squatting and jumping; and 3) thickening of the proximal part of the patellar tendon with areas of hypoechoic signals (Kulig et al. 2013).

All of the subjects filled in a form recording their age, weight, height, and training duration per week and completed the Victorian Institute of Sport Assessment-patella (VISA-P) questionnaire.

Figure 6.1 shows the study flow. The subjects were randomized into treatment and sham groups by drawing cards. Tendon and muscle stiffness, the intensity of pressure pain, and squatting pain during a single-legged decline squat test (SLDST) were evaluated before and immediately after intervention.

6.3.2 Assessment of the elastic properties of the patellar tendon and quadriceps muscle components

The procedures for evaluating the stiffness of the patellar tendon and thigh muscles were the same as those described in Chapters 2 and 4, respectively.

6.3.3 Pressure pain evaluation

Pressure pain was measured using the same instrument and following the same procedure as delineated in Chapter 5. In brief, the pressure pain induced by an algometer was assessed by the subjects using a visual analogue scale (VAS).

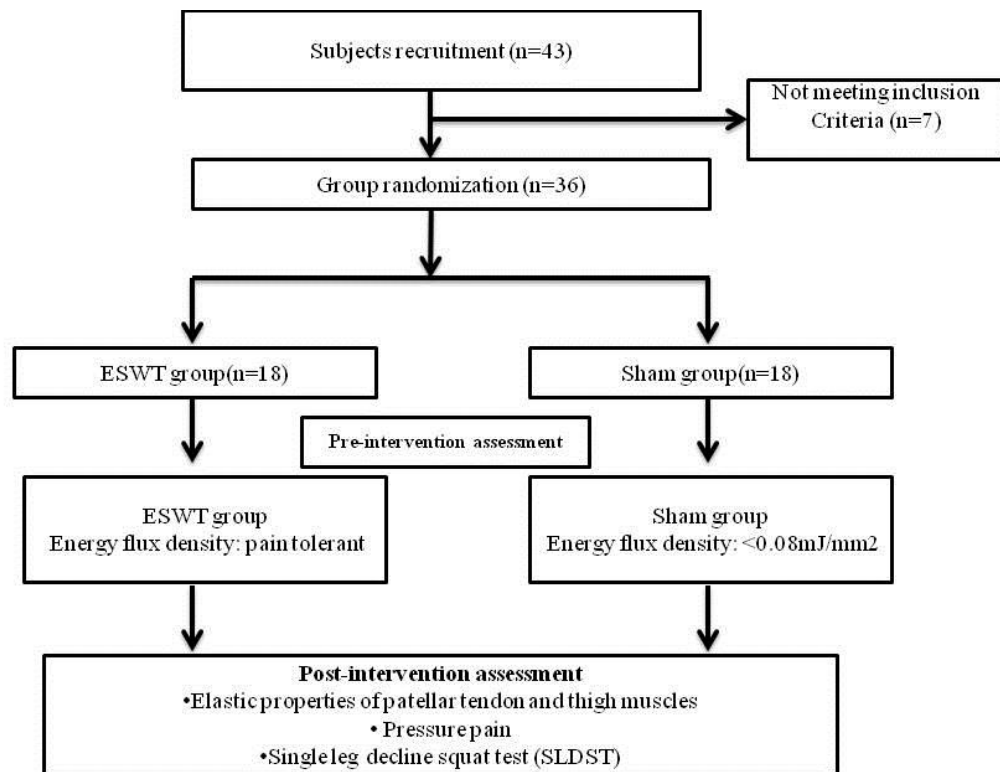


Figure 6.1 Flow chart of the experimental procedure.

6.3.4 Single-legged declined- squat test

Single-legged decline squat tests were conducted (Kongsgaard et al. 2006; Zwerver et al. 2007). The angle of knee flexion when pain was first perceived and the intensity of the first perceived pain were assessed. The subjects were required to stand single-legged on a 25-degree decline board, and were instructed to flex the knee until pain was elicited (Fig.6.2). The angle of the knee was measured by a goniometer ($SLDST_{\text{angle}}$) and the magnitude of pain was reported using the VAS ($SLDST_{\text{pain}}$).

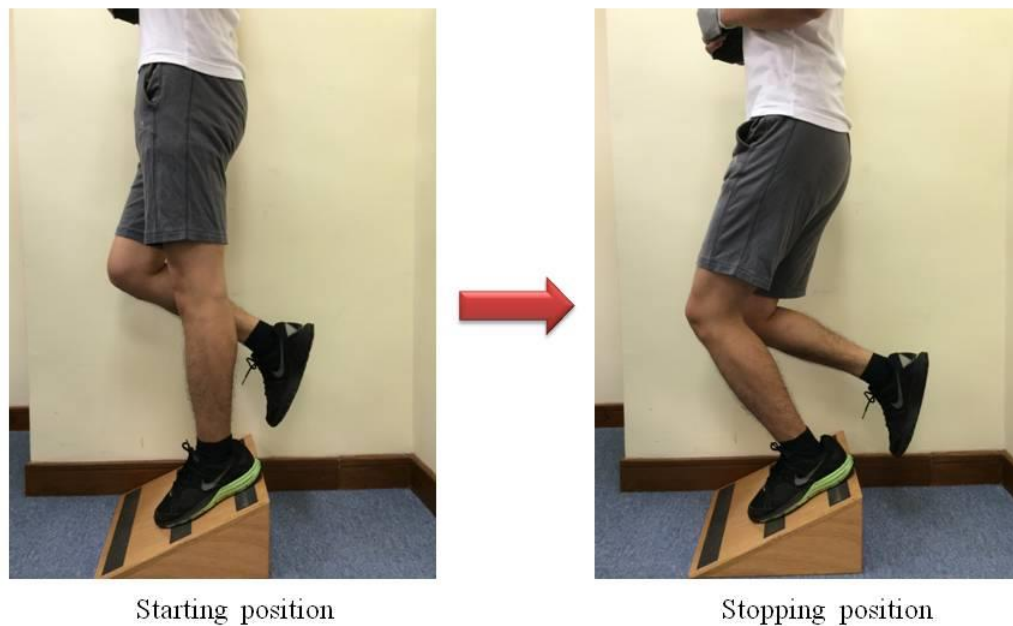


Figure 6.2 Single- legged declined- squat test (A: starting position; B: stop position when pain eliciting)

6.3.5 Extracorporeal shock wave intervention

ESWT was delivered by an experienced physical therapist at the Rehabilitation Clinic of the Hong Kong Polytechnic University. The subjects were positioned in supine lying with the treated knee supported at 30 degrees of flexion. The most painful spot on the patellar tendon was palpated and marked. Focused ESWT was delivered by a Storz Minilith SL1 lithotripter machine (Storz Medical, Switzerland) at the marked spot (Fig. 6.3). Contact gel was applied between the patellar tendon and the applicator to minimize the loss of shock wave energy. In the treatment group, 1500 impulses at maximum tolerable pain were delivered at 4 Hz (Vetrano et al. 2013). A low energy treatment (less than $0.08\text{mJ}/\text{mm}^2$) was delivered at the same frequency and with the same number of impulses to the sham group. For subjects with bilateral symptom, the more painful side was selected for intervention.



Figure 6.3 Position of the patient and the shockwave head of the extracorporeal shock wave therapy for delivering to the patellar tendinopathy.

6.3.6 Statistical analysis

Descriptive statistics (mean and standard deviations, percentages) were used to describe the physical characteristics of the ESWT and sham groups, and the outcome variables before and after intervention. The percentage changes in the tendon and muscle shear elastic moduli, pressure pain, $SLDST_{\text{pain}}$, and $SLDST_{\text{angle}}$ were computed. Composite change on pain and knee angle were calculated by summation of $SLDST_{\text{pain}}$, and $SLDST_{\text{angle}}$ and expressed as $SLDST_{\text{pain_angle}}$. The data normality of these variables was assessed using the Shapiro-Wilk test. An independent t-test was used to compare the demographic data of the ESWT and sham groups. Univariate analysis of variance tests were used to assess the between group differences in the percentage changes in the tendon and muscle shear elastic moduli, pressure pain, $SLDST_{\text{pain}}$, and $SLDST_{\text{pain_angle}}$ in the treated legs. Between-leg differences were assessed using univariate analysis of variance tests on the outcome variables within each group. Pearson correlation coefficient tests were conducted to examine the relationships among outcome variables. SPSS version 17.0 (SPSS Inc, Chicago, IL) was used to perform the statistical analyses. The level of statistical significance was set at $p < 0.05$.

6.4 Results

6.4.1 Participant's demographic data

A total of 43 subjects were recruited for this study. The VAS scores of seven subjects had reduced to less than 3 when they attended the treatment session. The age, height, weight, BMI training intensity, and pain duration of the subjects are shown in Table 6.1. No significant group differences were found in age, BMI, training history,

training intensity, pain duration, pain intensity, or the VISA scores ($p>0.05$). In the ESWT group, the treatment intensity ranged from 0.13mJ/mm² to 0.33mJ/mm². All but one subjects had treatment intensities below 0.28mJ/mm².

Table 6.1. Subjects characteristics

Variables	ESWT group (n=18)	Sham group (n=18)	<i>P</i> Value
Age (y)	21.6±3.0	23.4±4.6	0.205
Weight (kg)	76.2±6.3	72.9±5.7	0.106
Height (cm)	182.2±5.7	178.3±6.4	0.059
Body mass index (kg/m ²)	22.9±1.5	22.9±2.1	0.936
Training intensity (hours/w)	6.4±3.4	4.9±2.2	0.140
Training years (y)	8.1±2.4	9.6±4.2	0.215
Duration of symptom (months)	34.7±22.4	28.5±27.0	0.461
Baseline scores			
VAS	7.0±1.7	6.7±1.6	0.528
VISA	54.6±14.3	58.1±9.4	0.392

Values are reported as mean±standard deviation. VAS: visual analogy scale; VISA: Victorian Institute of Sport Assessment

6.4.2 Treatment effects

Significantly greater reduction on patellar tendon and VL muscle stiffness were observed in the ESWT group when compared with the sham groups (Table 6.2, all $p<0.05$). More specifically the tendon shear elastic modulus was reduced from 54.1kPa to 38.8kPa in the ESWT group; and from 48.6kPa to 42.2kPa in the sham group. The muscle stiffness of the VL was reduced from 4.9kPa to 3.9kPa in the ESWT group; but was from 4.6kPa to 4.1kPa in the sham group. No significant group differences were

detected on the RF muscle stiffness. No significant changes on tendon and muscle shear elastic modulus were observed in the non-treated leg (Table 6.2).

No significant group difference on the percentage changes on pressure pain was observed. The percentage changes on pressure pain were 14.2% and 9.2% in the ESWT and sham groups, respectively (Table 6.2, $p>0.05$). In addition, there was no significant difference in SLDST_{pain} and SLDST_{pain_angle} between in the ESWT group and in the sham group (Table 6.2, $p>0.05$).

Table 6.2 Percentage changes on tissue elasticity and intensity of pressure pain and activity-related pain

Variables	Treated leg		<i>p</i>	Non-treated leg		<i>P</i>
	ESWT group (n=18)	Sham group (n=18)		ESWT group (n=18)	Sham group (n=18)	
Patellar tendon (%)	24.7±17.6	8.0±24.0	0.027	-1.2±9.3	2.4±14.3	0.392
VL muscle (%)	18.8±10.5	10.6±12.5	0.041	-2.0±9.5	-6.6±17.0	0.334
RF muscle (%)	-3.5±25.1	-1.2±18.0	0.377	-0.5±17.7	-7.9±31.2	0.398
Pressure pain (%)	14.2±18.7	9.2±24.2	0.495	----	----	----
SLDST _{pain} (%)	2.8±25.4	14.2±30.1	0.228	----	----	----
SLDST _{pain_angle} (%)	-1.9±12.5	-4.2±11.8	0.566	----	----	----

Values are reported as mean+standard deviation. VL: vastus lateralis; RF: rectus femoris. % change after intervention=(pretest-posttest)/(pretest)×100%

Table 6.3 Percentage changes on tissue elasticity after ESWT

Variables	ESWT group		<i>p</i>	Sham group		<i>P</i> Value
	Treated leg	Non-treated leg		Treated leg	Non-treated leg	
Patellar tendon (%)	24.7±17.6	-1.2±9.3	0.000	8.0±24.0	2.4±14.3	0.397
VL muscle (%)	18.8±10.5	-2.0±9.5	0.000	10.6±12.5	-6.6±17.0	0.001
RF muscle (%)	-3.5±25.1	-0.5±17.7	0.685	-1.2±18.0	-7.9±31.2	0.434

Values are reported as mean+standard deviation. VL: vastus lateralis; RF: rectus femoris. % change after intervention=(pretest-posttest)/(pretest)×100%

6.4.3 Relationship among percentage changes in patellar tendon shear elastic modulus, thigh muscle shear elastic modulus, pressure pain, single-legged declined-squat pain and range

Table 6.4 shows the correlations between the outcome variables in the ESWT and sham groups. In the ESWT group, the percentage change in the tendon shear elastic modulus was related to the percentage change in the intensity of pain during SLDST ($r=0.52$, $p=0.04$) (Table 6.4) and the percentage change in the intensity of pain and knee range during SLDST ($r=0.59$, $p=0.019$) (Table 6.4). In other words, a greater reduction in the tendon elastic modulus was associated with a greater reduction in pain and composite reduction on pain and knee range during SLDST. In the sham group, the percentage change in the patellar shear elastic modulus was related to the percentage change in the VL shear elastic modulus ($r=0.62$, $p=0.006$) (Table 6.4).

Table 6.4 Pearson correlations between percentage changes of patellar tendon shear elastic modulus and variables

Variables	ESWT group	Sham group	All
VL muscle	0.16	0.62*	0.54*
RF muscle	0.04	0.10	0.07
Pressure pain	0.05	0.27	0.18
SLDST_pain	0.52*	0.24	0.25
SLDST_angle	0.07	0.04	0.03
SLDST_angle+pain	0.59*	0.20	0.21
EFD	0.00		

* $P<0.05$; VL: vastus lateralis; RF: rectus femoris; EFD:energy flux density, all variables: % changes after intervention.

6.5 Discussion

In this study, a session of ESWT was found to induce a reduction in the tendon and muscle stiffness in male jumping athletes with patellar tendinopathy. A greater reduction in tendon stiffness was associated with a greater reduction in the intensity of pain during the single-legged decline squat test. This relationship was not observed in the sham group or the untreated leg of the ESWT group.

The immediate effects of a single session of focused ESWT on the elastic properties of the patellar tendon were examined. The results revealed that the patellar tendon shear elastic modulus was significantly reduced by 24.7% in the ESWT group. This is an important finding because increased tendon stiffness is associated with self-perceived pain in subjects with PT (Zhang et al. 2014). Changes in tissue compliance have been observed after therapeutic ultrasound on the skin (Dinno et al. 1989), muscle (Daraper et al. 2010), and tendon (Lehmann et al. 1970). In these studies, the observed increase in tissue compliance was attributed to the thermal effects induced by the ultrasound. The increase in tissue temperature may lead to an increase in tissue circulation and viscoelasticity. Extracorporeal shockwaves are acoustic waves but have greater peak pressure than therapeutic ultrasound. Accordingly, an increase in tissue temperature may have been induced at the treated tendon, leading to the observed reduction in tendon stiffness. Further research is required to assess the temperature change induced by ESWT. In addition, the higher pressure stimulus induced by ESWT on the patellar tendon may have induced a greater reduction in the H-reflex, muscle tone (Kukulka et al. 1985), and motor nerve extensibility (Bae et al. 2010) compared with the

sham group. The changes in muscle tone and muscle activation may have indirectly affected the tendon stiffness.

Indeed, a significant reduction in VL muscle stiffness was observed in the ESWT group (by 18%) and in the sham group (by 10%), but showed minimally change in the untreated leg (by -2% to -6.6%). These findings suggested that the pressure stimulus induced by the low energy ESWT in the sham group may have induced a reduction in the resting muscle tone via its influence on the H-reflex (Kukulka et al. 1985). The ESWT group received greater pressure stimulus and pain at the maximum tolerable intensity. Pain may also induce a reduction in muscle activity and alter the resting stiffness of the muscles. Using similar technologies (SSI), Hug et al. (2014) reported that a reduction in RF muscle stiffness during muscle contraction when pain was experimentally induced by the injection of hypertonic saline in the RF muscle. In most of the previous studies, EMGs were used to assess the modulation of muscle activities after experimentally induced pain. Graven-Nielsen et al. (1997) reported that the EMG activity of the tibialis anterior muscle was reduced during both static and dynamic contraction after an injection of 0.5 ml hypertonic saline (5%) into the muscle belly. Madeleine (2006) assessed the EMG activity of the upper trapezius muscle before and after 0.5 ml injection of hypertonic saline (5.8%). They also observed a decrease in the muscle activity of the upper trapezius muscle after the injection. The reduction in muscle activity induced by experimentally induced pain may be due to a decrease in the firing rate of the muscle alpha-motoneurons (Kniffki et al. 1981) or a reduction in the extensibility of the muscle spindles (Mense and Skeppar et al. 1991). The findings from the present study demonstrated that VL muscle stiffness was reduced in the ESWT and sham groups and

the magnitude of change was significantly greater in the ESWT group. The change in VL muscle stiffness may be related to the mechanical stimulus of the ESWT in the treated and sham groups, and the pain-induced modulation of muscle activity in the ESWT group.

The question remains whether the change in tendon stiffness was related to the reduction in the intensity of the activity-related pain. The findings of this study indicated that the reduction in stiffness of the patellar tendon was related to the reduction in pain during squatting, in that a greater reduction in the stiffness of the patellar tendon was correlated with a greater reduction in pain during squatting. The possible relationship between the change in muscle stiffness and pain was assessed by Maher et al. (2013). They assessed the stiffness of the upper trapezius muscle before and after dry needling at the muscle myofascial trigger points. In contrast to the findings of this study, the authors could not establish a relationship between the change in muscle stiffness and pain. In this study, the relationship between the change in tendon compliance and activity pain was assessed. After a session of ESWT, the change in the compliance of the patellar tendon was found to be positively related to the change in pain during single-leg squatting. The greater compliance of the patellar tendon indicated less pain during single-leg squatting.

The treatment efficacy of ESWT has been reported in patients with PT and Achilles tendinopathy (Al-Abbad and Simon 2013) in the lower extremity, and with supraspinatus tendinopathy (Huisstede et al. 2011) in the upper extremity. However, the underlying mechanism of ESWT remains unclear (Wang et al. 2012). An increase in collagen synthesis was observed after extracorporeal shockwaves with 1500 impulses at 0.29mJ/mm^2 were applied to PT in a rabbit model (Hus et al. 2004). Similarly, an increase in growth factors was reported by Chen et al. (2004). The authors observed an

increase in transforming growth factor-beta 1 (TGF- β 1) and insulin-like growth factor-I (IGF-I) after extracorporeal shockwaves were delivered at 0.16mJ/mm² with 200 impulses. Increased vascularization was found in healthy animals four weeks post intervention (Wang et al. 2002). In contrast, an immediate reduction in vascularization was found in patients with chronic supraspinatus tendinopathy (Notarnicola et al. 2011). In addition, pain reduction may be related to a decrease in nerve conduction (Ohtori et al. 2001; Wu et al. 2008) or the suppression of substance P and glutamine (Maier et al. 2003). The findings from this study disclosed a new treatment mechanism induced by ESWT. The changes in tendon mechanical properties induced by the extracorporeal shockwaves were found to be related to the perceived intensity of pain during a single-legged declined- squat test. The ESWT induced change in the tendon shear elastic modulus may be associated with the pressure effect of the shockwaves and/or the motor and neural consequences of the intense pain during the intervention.

In this study, no significant treatment effects of pressure pain were detected. Zwerver et al. (2011) also detected no significant difference in the pain from different functional activities (jumping/ squatting) after six sessions of ESWT on the patellar tendon. They proposed that the lack of significant findings was related to the subjects' continued participation in sport training. However, Wang et al (2007) found a significant decrease in self-perceived pain while walking up and down stairs after one session of ESWT on the patellar tendon.

ESWT is a safe and promising modality for reducing pain and improving function for subjects with PT. However, the most effective protocol has not been established (van Leeuwen et al. 2009; van der Worp et al. 2013). Treatment intensity is one the

determining factors. Medium intensity (ranging from 0.08mJ/mm² to 0.28mJ/mm²) but not high intensity (above 0.28mJ/mm²) ESWT was found to be effective in treating PT (Rompe et al. 1998; Wang et al. 2007; Taunton et al. 2007; Zwerver et al. 2011). In addition, the efficacy of ESWT was found to be greater when prescribed at the maximum tolerable pain level (Chow et al. 2007). In this study, the maximum tolerable pain was used as the criteria for treatment intensity. The treatment intensities for the majority (over 90%) of the subjects were 0.13 mJ/mm² and 0.17mJ./mm². However, it could not be established whether the treatment dosage had any effects on tendon and muscle stiffness. Further research is needed to explore whether the changes in tendon and muscle stiffness are associated with the treatment dosage. Although ESWT was applied at the maximum tolerable pain intensity, no subjects suffered edema or intense pain post intervention.

6.6 Scientific and clinical implications

Changes in tissue stiffness were observed after one session of ESWT on the treated (the tendon) and non-treated (the muscle) sites. The findings indicated that the ESWT induced local effects on the patellar tendon and distant effects on the VL muscle. Hence, aside from inducing local stimulation of the tendon, motor/neural consequences may have led to the change in VL stiffness. The findings also indicated that the reduction in the stiffness of the patellar tendon was associated with the reduction in activity-related pain. In addition to ESWT, other therapeutic interventions that can reduce tendon stiffness could be considered for treating PT. The change in tendon stiffness could also be used in assessing the treatment efficacy of different interventions, such as platelet-rich plasma.

6.7 Limitations

The findings from this study can only be generalized to male athletes involved in jumping sports. The cumulative effects of ESWT on tendon compliance and their relationship with treatment efficacy were not assessed. Three to five sessions of ESWT are commonly recommended for treating PT. However, it is not known whether the difference in tendon stiffness would be increased with repeated applications of ESWT. This study aimed to evaluate the local and neural post intervention effects of one session of ESWT. Long-term follow-ups are also important in assessing the treatment efficacy of ESWT. A minimum of three months is generally used in assessing the treatment efficacy of ESWT (Wang et al. 2007; Tanunton et al. 2003). Treatment efficacy has also been assessed one year after ESWT. Further research is needed to examine the cumulative effects of ESWT and the treatment efficacy at 3 to 12 months post treatment.

6.8 Conclusions

The findings of this study demonstrated that a single session of ESWT delivered at the maximum tolerable pain induced significant reductions in tendon stiffness and VL muscle stiffness. The reduction in tendon stiffness was associated with the subjects' self-perceived pain and overall performance during the single-legged declined-squat test. Overall, the findings suggest that the modulation of the tendon mechanical properties may be one of the treatment mechanisms of ESWT.

CHAPTER 7

SUMMARY AND CONCLUSIONS

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7.1 Rationale of the study

The elastic properties of the patellar tendon are essential for shock absorption on landing from a jump and for the storage of kinetic energy for subsequent jumps (Alexander et al. 2002; Arampatzis et al. 2006; Kubo et al. 2010; Magnusson et al. 2003). A tendon with more compliance can store and release more energy, allowing higher jump heights to be achieved (Kubo et al. 1999; Walshe and Wilson et al. 1997). It is therefore important to explore the effects of loading and overloading on tendon elasticity for performance enhancement and injury prevention.

Sports participation, in particular jumping sports, induces mechanical loading on the patellar tendon such that adaptation can occur. Volleyball and basketball are two jumping sports with different physical demands (Kollias et al. 2004; Laffaye et al. 2007). A comparison of tendon elasticity between sedentary individuals, volleyball players, and basketball players would provide an insight into the sports-specific loading on the tendon mechanical properties.

The hip muscles, in addition to sharing the landing impact (Decker et al. 2003), also control the landing kinematics (Jacobs et al. 2007). The alteration of landing kinematics increases the landing load on the patellar tendon and is one of the factors that causes tendinopathy (Edward et al. 2010). To our knowledge, there are no reported studies investigating hip muscle strength in jumping athletes with patellar tendinopathy and its correlation with tendon elasticity. The findings from this study will shed light on the effects of overloading on tendon elasticity and provide scientific evidence for injury prevention programs.

Overloading on the patellar tendon has been proposed as the causative factor in the development of PT (Lian et al. 1996). Changes in collagen orientation (Astrom et al. 1995; Kalebe et al. 1991), tendon thickness (Cook et al. 2000; Kulig et al. 2013), and cross-section (Gisslén and Alfredson et al. 2005) are likely to change the elastic properties of the affected tendon. However, no statistically significant difference in tendon elasticity was detected between athletes with and without PT (Couppe et al. 2013). Using state-of-the art equipment, tendon elasticity at the site of pain can be measured and related to the magnitude of pain. Such an approach may unveil regional changes in tendon elasticity associated with tendinopathy and how such changes are associated with the magnitude of self-perceived pain.

Extracorporeal Shockwave Therapy (ESWT) is a safe and promising method of reducing pain and improving function in individuals with PT (van der Worp et al. 2013). Aside from inducing mechanical effects at the treated site, the associated pain can induce alterations in the muscle activities in the quadriceps muscle (Hodges et al. 2009). Either or both effects may lead to a change in tendon elasticity. The association between tendon elasticity and pain can be established further if ESWT-induced changes in pain are shown to be associated with changes in tendon elasticity.

Therefore, this thesis's five interrelated studies had the following aims.

Study 1: Assess the correlation of the shear elastic modulus captured using Supersonic Shearwave Imaging (SSI), the tangent traction modulus using a Material Testing System, and the test-retest reliability of the shear elastic modulus captured from the SSI of the patellar tendon.

- Study 2: Compare the elastic properties of the patellar tendon between sedentary subjects, volleyball players, and basketball players and examine the correlation between the tendon elastic properties and age, body mass index, and training intensity among players.
- Study 3: Compare the isometric muscle strength of the hip abductors and external rotators in athletes with and without PT and explore the possible association between the hip muscle strength and tendon elastic properties.
- Study 4: Explore changes in the elastic properties of the patellar tendon in athletes with unilateral PT and examine associations between changes in tendon shear elastic modulus, pain, and dysfunction in athletes with unilateral PT.
- Study 5: Determine the effects of a single session of ESWT on the elastic properties of the quadriceps muscle and proximal patellar tendon and the magnitude of pain in athletes with PT.

7.2 Summary of the studies

7.2.1 Study 1. Elastic modulus on the patellar tendon captured using SSI: correlation with the tangent traction modulus computed using a material testing system and test-retest reliability

Eight fresh patellar pig tendons were used to examine the correlation between the shear elastic modulus on the patellar tendon captured using SSI (Aixplorer® ultrasound unit V4, Aix-en-Provence, France) and the tangent traction modulus computed using a material testing system. The testing procedures were described in Chapter 2. A good to

excellent correlation coefficient between the shear elastic modulus from the SSI and tangent traction modulus from the MTS was 0.82 to 1.00 ($p<0.05$) In addition, excellent intra- and inter-operator reliability of the shear elastic modulus captured using the SSI was established on 22 patellar tendons of 11 healthy human subjects (all ICC >0.90). The minimum detectable difference of the elastic modulus of the patellar tendon was 4.27 kPa.

The findings of this study indicate that SSI is a reliable tool for measuring the mechanical properties of the patellar tendon. In addition, any difference in shear elastic modulus greater than 4.27 kPa can be interpreted as a true difference.

7.2.2 Study 2. Elastic properties of the proximal patellar tendon: effects of age, BMI, leg dominance, and sports participation

Fifty healthy male subjects aged between 18 and 35, consisting of 20 sedentary subjects, 15 volleyball players, and 15 basketball players, were recruited. SSI was used to measure the elastic properties of the proximal patellar tendon on the dominant and non-dominant knees. The volleyball players had the lowest tendon shear elastic modulus when compared with the basketball players (by 24.9%, $p<0.05$) and sedentary subjects (by 42.3%, $p<0.05$). The basketball players had a significantly lower tendon shear elastic modulus when compared with the sedentary group (by 23.1%, $p<0.05$). No significant side-to-side difference in the tendon shear elastic modulus was detected ($p<0.05$). A positive correlation was detected between the patellar tendon shear elastic modulus and age in the volleyball group ($r=0.53$; $p=0.003$).

The results from this study suggest that habitual loading induces an adaptation of the patellar tendon elasticity and that the adaptation is sport-specific. However, there is

no side-to-side dissymmetry in tendon elastic properties at the proximal patellar tendon in healthy sedentary individuals and athletes. In addition, age seemed to reduce tendon compliance in the volleyball players.

7.2.3 Study 3. Hip strength in male players with and without PT and its association with the elastic properties of the patellar tendon

Sixty-six male athletes (mean age of 21.1 ± 4.4 years) participated in this study, with 33 subjects diagnosed with PT for more than 3 months. The muscle strength of the hip abductors and external rotators was quantified using a hand-held dynamometer. The elastic properties of the patellar tendon and the vastus lateralis and rectus femoris muscles were assessed using SSI technology.

No significant side-to-side differences were detected in the hip muscle strength and the elastic shear modulus of the tendon and muscles in the control group (all $p > 0.05$). The normalized isometric hip muscle strength was found to be significantly weaker (by 11.2% to 18.2% in the external rotators and abductors, respectively; all $p < 0.05$), and the shear elastic modulus was significantly increased in the patellar tendon (by 48%) and in the vastus lateralis (by 26.5%) in players with PT, when compared with the healthy controls. A significant correlation was detected between the tendon shear elastic modulus and normalized isometric muscle strength of the hip muscles.

This study demonstrated that players with PT had lower strength of muscle on their hip abductors and external rotators. Weakness in these hip muscles was associated with a reduction in patellar tendon elasticity. Such findings suggest that hip muscle strength is one of the factors affecting tendon compliance. In addition, training of the hip

muscles might reduce the incidence of PT for athletes involved in volleyball and basketball.

7.2.4 Study 4. Changes in the morphological and elastic properties of the patellar tendon in athletes with unilateral PT and their relationships with pain and functional disability

Thirty-three male athletes (20 healthy and 13 with unilateral PT) were enrolled. The morphology and elastic properties of the patellar tendon were assessed by the grey and elastography mode of SSI. The intensity of pressure pain, self-perceived pain, and dysfunction were quantified with a 10 lb force to the most painful site and the Victorian Institute of Sport Assessment-patella (VISA-p) questionnaire.

The results showed that the painful tendons had a higher shear elastic modulus and were larger than the non-painful side ($p < 0.05$) or the dominant side of the healthy athletes ($p < 0.05$). Significant correlations were found between the tendon shear elastic modulus ratio (painful over non-painful tendon) and the intensity of pressure pain ($\rho = 0.62$; $p = 0.024$), VISA-p scores ($\rho = -0.61$; $p = 0.026$), and the sub-scores of the VISA-p scores when going down stairs, lunging, single leg hopping, and squatting (ρ ranged from -0.63 to -0.67 ; $p < 0.05$).

This study revealed changes in both morphology and elastic properties at the painful part of the patellar tendon in athletes with unilateral PT. The affected tendons were stiffer, thicker, and had a larger cross-sectional area than the non-painful side and the tendons of the healthy controls. In addition, the ratio of the painful and non-painful

tendon shear elastic properties was associated with the intensity of pressure pain and VISA-p scores in athletes with unilateral PT.

7.2.5 Study 5. Immediate effects of one session of ESWT on the tendon shear elastic modulus in athletes with chronic PT

Thirty-six male athletes aged between 18 and 32 with PT for more than 3 months (ranging from 3 to 72 months) were recruited. They were randomly assigned to the ESWT or control groups. Players in the ESWT group received a session of ESWT at their maximum tolerable pain level at 4 Hz for 1500 impulses. The minimum treatment intensity (less than 0.08 mJ/mm²) at 4 Hz for 1500 impulses was applied to the players in the control group. SSI was used to measure tendon and muscle stiffness. A hand-held algometer was used to apply a 10 lb pressure at the tender spot of the affected tendon. The intensity of the pressure pain was quantified using the visual analogy scale (VAS). The single-legged declined-squat test (SLDST) was used to assess the angle of knee flexion when the pain was first perceived and the intensity of the first-perceived pain (based on the VAS). All of these outcome measures were conducted before and immediately after the application of ESWT.

A significantly greater reduction in the tendon shear elastic modulus was detected in the ESWT group when compared with the control group ($p < 0.05$). In the ESWT group, the change in the tendon shear elastic modulus was related to the change in the intensity of squatting pain and the composite change in the knee range and squatting pain ($r = 0.52$ and 0.59 , respectively; all $p < 0.05$). In the control group, the change in the tendon shear elastic modulus was related to the change in the shear elastic modulus of the vastus

lateralis muscle ($r=0.62$, $p=0.006$). No significant changes in the tendon and muscle shear elastic modulus were detected in the non-treated leg in the ESWT and control groups.

Our findings demonstrated that a single session of ESWT delivered at maximum tolerable pain induced significant reduction in tendon stiffness. The reduction in tendon stiffness was associated with self-perceived pain and composited change in knee range and pain during the SLDST. These observations further confirm the association between tendon elasticity and self-perceived pain in patients with PT. Modulation of the tendon's mechanical properties may also be one of the treatment mechanisms of ESWT.

7.3 Limitations and future studies

Only male subjects were recruited in this study. The findings from this study cannot be generalized to female subjects. A similar study on female athletes is strongly suggested to extend our observations. The elastic properties of the patellar tendon were evaluated at its proximal portion. Loading effects induced from sports participation and muscle weakness on the whole tendon or at other parts of the tendon could not be established. We took measurements at the proximal patellar tendon because that is where most pathological changes and pain occurred in patients with PT. Further exploration could be conducted to compare regional differences between jumping athletes with and without PT. In addition, weakness in the hip muscles and tendon elasticity are related. Whether such an association is due to a shift in load sharing or an alteration in the landing kinematics awaits further exploration. Despite an observed increase in the tendon shear elastic modulus in players diagnosed with tendinopathy, the causes of such a change have not been established. Further experiments can be conducted to correlate the

findings from the SSI and histological tests. Lastly, we observed an immediate reduction in the tendon elasticity after a session of ESWT on the patellar tendon. The long-term effects of ESWT pain in patients with chronic PT are suggested to explore the treatment efficacy and mechanism of ESWT.

7.4 Significance of the project

The findings of this project demonstrate that SSI is a reliable method to quantify the elastic properties of the patellar tendon. Using this technique, localized elastic properties can be assessed in regions where pathology and pain occur.

Second, our findings suggest that the elastic properties of the patellar tendon can be modulated with habitual loading. The intensity of modulation seems to be sports specific. More specifically, the patellar tendon has better compliance in individuals participating in sports with greater demands for jump-land activities.

Third, weaker muscle strength in the hip abductors and external rotators was observed in those players with PT. Such information indicates that strengthening programs targeting the hip abductors and external rotators is recommended for the prevention of PT for athletes involved in volleyball and basketball.

Fourth, athletes with PT had stiffer tendons when compared with healthy controls. More importantly, tendon stiffness was associated with self-perceived pain. This observation suggests that tendon stiffness is one of the causative factors of pain in individuals with PT.

Lastly, an increase in tendon elasticity was observed after a session of ESWT. The reduction in tendon stiffness is associated with a reduction in activity-related pain. These findings further confirm the relationship between tendon stiffness and pain associated with PT. Modulation of the tendon's mechanical properties may be one of the mechanisms of ESWT.

7.5 New contributions of the projects

1) Established the validity and test-retest reliability of SSI in quantifying regional tendon elastic properties;

2) Identified modulation on patellar tendon elastic modulus associated with habitual loading and overloading-induced tendinopathy;

3) Related intrinsic factors such as hip muscle strength and quadriceps muscle stiffness with patellar tendon stiffness;

4) Confirmed relationship between patellar tendon stiffness and activity-related pain;

5) Information generated could be used for prevention and rehabilitation of patellar tendinopathy for volleyball and basketball players.

APPENDIX I



THE HONG KONG
POLYTECHNIC UNIVERSITY
香港理工大學

MEMO

To : FU Stu Ngor, Department of Rehabilitation Sciences

From : YIP Kam Shing, Chairman, Faculty Research Committee, Faculty of Health & Social Sciences

Ethical Review of Research Project Involving Human Subjects

I write to inform you that approval has been given to your application for human subjects ethics review of the following research project for a period from 16/02/2011 to 01/01/2014:

Project Title : The effects of extracorporeal shockwave therapy on physiological and mechanical properties of patellar tendon in patients with patellar tendinopathy

Department : Department of Rehabilitation Sciences

Principal Investigator : FU Stu Ngor

Please note that you will be held responsible for the ethical approval granted for the project and the ethical conduct of the research personnel involved in the project. In the case the Co-PI 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 Faculty Research Committee Faculty of Health & Social Sciences in advance of any changes in the research proposal or procedures which may affect the validity of this ethical approval.

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

YIP Kam Shing

Chairman

Faculty Research Committee

Faculty of Health & Social Sciences

APPENDIX II

香港理工大學康復治療科學系科研同意書

科研題目：體外衝擊波對髕骨筋腱炎患者的筋腱生理變化及結構屬性的影響

科研人員：張志傑, 李偉俊（香港理工大學康復治療科學系博士生）

Mobile: 6570

導師：

符少娥博士 (香港理工大學康復治療科學系副教授)

吳賢發教授 (香港理工大學康復治療科學系系主任及講座教授)

科研內容：

髕骨筋腱炎位列跳躍性運動員膝部問題之首。當其症狀成為頑固的長期問題時,往往不能有效地根治。有臨床實驗證據顯示體外衝擊波對此問題有一定治療效果。而在動物實驗中亦顯示體外衝擊波能促進筋腱患處的再生能力,從而加強筋腱結構屬性。本研究目的為體外衝擊波在人體的治療機理提供醫學證據。本科研分兩部份,參加者可能被安排完成第一部份或全部。

科研目標：

- (1)了解運動員髕骨筋腱的健康狀況和結構屬性的關係
- (2)檢測體外衝擊波對髕骨筋腱炎患者的筋腱結構屬性的潛在變化
- (3)分析髕骨筋腱炎患者的筋腱結構屬性、臨床表現和客觀效能之變化的關係

科研方法與程序：

第一部份：本研究將在香港理工大學進行,獲邀請的參加者將會作以下檢測,時間大約一至兩小時。

- 填寫問卷，內容包括練習時間、整體健康狀況及過去六個月的創傷等
- 膝部臨床檢驗(如需要)
- Victorian Institute of Sport Assessment (VISA) 問卷 (如需要)
- 以肌力測試儀及超聲波影像檢測髌骨韌帶的結構屬性
- 斜板上單腿蹲
- 大腿前肌及後肌柔韌度
- 超聲波影像檢測於靜態和動態的大腿及髌骨筋腱
- 量度身高、體重

第二部份：本研究將在香港理工大學進行，獲邀請的參加者於膝痛患處將接受六次的體外衝擊波治療，每週一一次。期間，參加者會反覆作第一部份所做的檢測，檢測安排於第一次治療後的三、六及十二週進行。

2 可能產生之副作用、危險、不適及處理方法：

肌肉力量、單腿蹲及單腿跳遠測試有可機會導致延遲性肌肉酸痛或疲倦乏力。測試後作充足的休息可達到全面舒緩的效果。

接受體外衝擊波治療的參加者可能在治療時及治療後一至兩天患處會感到痛楚加重。根據本研究小組的經驗，這問題通常會在一一至兩天內消退。

本科研項目的益處：

是次研究項目的資料將有助深入了解體外衝擊波治療在處理髌骨筋腱炎的效用。亦從仔細分析髌骨筋腱炎患者的筋腱結構屬性、臨床表現和客觀效能之變化的關係，令醫療人員和科研人員加深對體外衝擊波治療機理的認識。

自願參與性質：

本研究全屬自願參與性質，閣下有權利於任何階段退出及中止參與是次研究。閣下之任何決定不會構成任何與香港理工大學康復治療科學系關係之改變。

機密性：

閣下所提供的任何資料將會被研究人員及其相關的醫療同仁保密。除非得到閣下的「授權」（允許），否則閣下的姓名或可用以直接查明本人身份的其他資料絕不會出現於印刷品、報告及期刊中。

閣下之資料保密性將受法律最全面的保障。

同意書：

本人_____已瞭解此次研究的具體情況。本人願意參加此次研究, 本人有權在任何時候、

無任何原因放棄參與此次研究, 而此舉不會導致我受到任何懲罰或不公平對待。本人明白參加此研

究課題的潛在危險性以及本人的資料將不會洩露給與此研究無關的人員，我的名字或相片不會出現

在任何出版物上。

本人可以用電話 **27666726** 來聯繫此次研究課題負責人，符少娥博士。若本人對此研究人員

有任何投訴，可以聯繫梁女士（部門科研委員會秘書），電話：**27665397**。本人亦明白，參與此研

究課題需要本人簽署一份同意書。

簽名（參與者）：日期: _____

簽名（證人）：日期： _____

APPEDIX III

The Hong Kong Polytechnic University

Department of Rehabilitation Sciences

Research Project Informed Consent Form

Project Title: The effects of extracorporeal shockwave therapy on physiological and mechanical properties of patellar tendon on patients with patellar tendinopathy

Investigator

Mr. Zhang Zhi Jie and Arthur Lee, PhD candidate, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University

Supervisor

Chief supervisor: Dr. Amy Fu, PhD, Associate Professor, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University

Co-supervisor: Prof. Gabriel Ng, PhD, Chair Professor & Associate Head, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University

Project information:

Introduction

Patellar tendinopathy (PT) is a major problem for many jumping athletes. Clinically, this problem is usually resistant to conservative treatment when becoming chronic in nature. Extracorporeal shockwave therapy (ESWT) appears to be a promising treatment with evidence of clinical trials. Animal studies exploring working mechanism suggested that ESWT might facilitate regeneration of pathological tendon, and hence improving the mechanical properties (MP). This study aims to examine whether this mechanism present *in vivo* human tendon. The research project divided into two studies, subjects may be arranged to participate in the first or both studies.

Research purposes

(1) To explore possible change in elastic properties the patellar tendon in athletes with chronic patellar tendinopathy.

(2) To investigate the effect of ESWT on mechanical properties of patellar tendon and clinical outcomes in athletes with chronic PT.

(3) To delineate possible relationship among elastic properties of tendon, pain, and functional performance in athletes with patellar tendinopathy.

Research method and procedures

Study 1: This examination carried in the polytechnic university. Invited subjects will be examined the followings, which last for 60-120 minutes.

- A questionnaire regarding your duration of training, general health condition and knee problems
- Clinical examination of knee (if applicable)
- Victorian Institute of Sport Assessment (VISA) questionnaire (if applicable)
- Mechanical properties testing using ultrasonography and dynamometry as well as shear wave imaging(detail fact sheet on the procedure will be given on the testing day)
- 2 test trials of single leg squat in decline board, single leg hop for distance
- 2 test trials of muscle length measurement of bilateral hamstrings and quadriceps muscles using passive knee extension and passive knee flexion tests with 5 seconds pause
- Ultrasound scanning of the quadriceps muscle and patellar tendon using a 12 MHz linear probe at rest and during contraction
- Height and weight measurement

Study 2: This examination carried in the polytechnic university. Invited subjects will receive 6 sessions of ESWT, with one in every week. Follow-up evaluations, as described in the study 1 will be carried at 3,6 and 12 weeks after the first treatment session.

Risks and Discomforts

There is possible of delay muscle soreness or fatigue after dynamometry, squat and hop test, sufficient rest afterwards should be able to eliminate the discomforts. Subjects receiving ESWT might experience increase in pain during treatment or 1-2 days after treatment. According to the research team's experience, the discomfort normally would subside in 1-2 days.

Benefits of the Research

This study aims to provide evidence on the treatment effectiveness in the management of PT using ESWT. In addition, clinician and researchers might enrich their understanding on the working mechanism of ESWT by knowing the interrelationship among tendon elastic properties, pain and functional performance *in vivo*.

Voluntary Participation

Your participation in the study is completely voluntary and you may choose to stop participating at any time. Your decision not to volunteer will not influence the nature of your relationship with Hong Kong Polytechnic University either now, or in the future.

Confidentiality

All information you supply during the research will be held in confidence and unless you specifically indicate your consent, your name or any identifiable information will not appear in any report or publication of the research. Confidentiality will be provided to the fullest extent possible by law.

Consent

I, _____, have been explained the details of this study. I voluntarily consent to participate in this study. I understand that I can withdraw from this study at any time without giving reasons. I am aware of any potential risk in joining this study. I also understand that my personal information will not be disclosed to people who are not related to this study and my name or photograph will not appear on any publications resulted from this study.

I can contact the investigator, Mr. Arthur Lee at telephone 9623 _____ for any questions about this study. If I have complaints related to the investigator(s), I can contact Mrs Michelle Leung, secretary of Departmental Research Committee, at 2766 _____. I know I will be given a signed copy of this consent form.

Signature (subject)

Signature (Witness)

Printed name(subject)

Printed name(Witness)

Date

Date

APPEDIX IV

膝髕腱疼痛問卷 (VICTORIAN INSTITUTE OF SPORT ASSESSMENT SCALE)

1. 你可以坐多長時間沒有膝痛？

	<i>10</i>											<i>0</i>			
疼痛程度															
0 分鐘													100 分鐘	右	分數
0 分鐘													100 分鐘	左	分數
	0	1	2	3	4	5	6	7	8	9	10				

2. 平常速度落樓梯時有膝痛嗎？

	<i>10</i>											<i>0</i>			
疼痛程度															
非常痛													無痛	右	分數
非常痛													無痛	左	分數
	0	1	2	3	4	5	6	7	8	9	10				

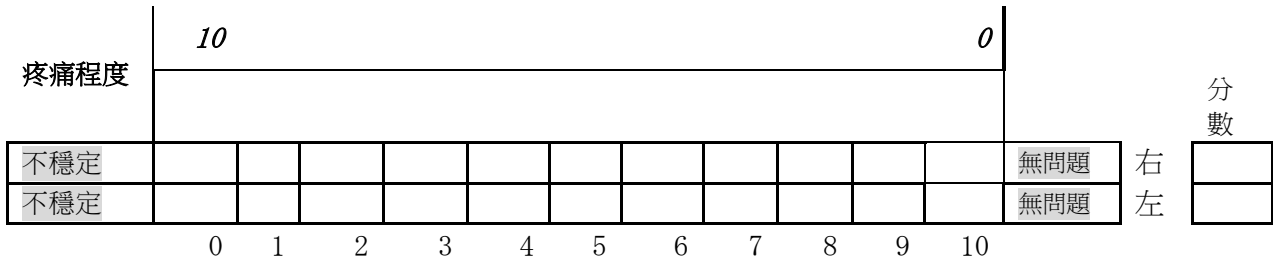
3. 坐著時主動伸直腳有膝痛嗎？

	<i>10</i>											<i>0</i>			
疼痛程度															
非常痛													無痛	右	分數
非常痛													無痛	左	分數
	0	1	2	3	4	5	6	7	8	9	10				

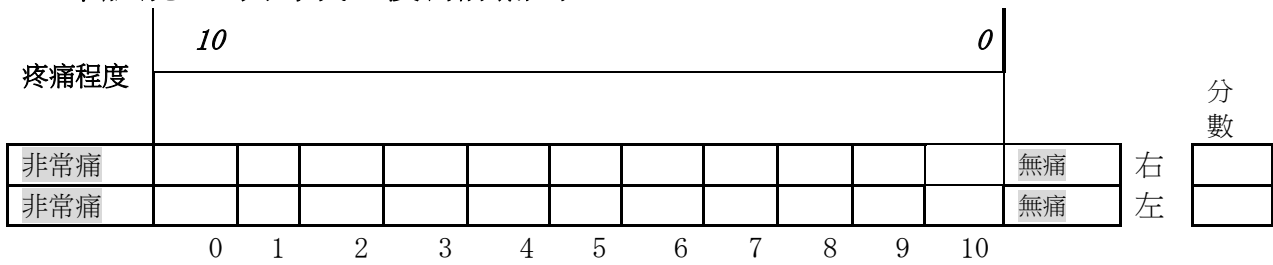
4. 弓步著地時有膝痛嗎？

	<i>10</i>											<i>0</i>			
疼痛程度															
非常痛													無痛	右	分數
非常痛													無痛	左	分數
	0	1	2	3	4	5	6	7	8	9	10				

5. 雙腿下蹲時有沒有問題？



6. 單腿跳 10 次時或之後有膝痛嗎？



7. 你現在可作那種程度的運動或活動？

0		不能運動；
4		做有限的訓練+比賽；
7		正常訓練+比賽，但跟膝痛之前強度不一樣；
10		能完成跟膝痛之前一樣或更高強度的比賽。

8. 以下問題，請回答 8a，8b 或 8c

- i 運動時沒有膝痛，請答 8a，
- ii 運動時有膝痛，但是痛不至於令你停止運動，請回答 8b，
- iii 運動時有膝痛，疼痛令你不得不停止運動，請回答 8c

8a. 如果運動時無膝痛，你可以訓練多長時間？

無	0-5 分鐘	6-10 分鐘	11-15 分鐘	> 15 分鐘	
0	7	14	21	30	分數

8b. 運動時有膝痛，但是不會令你停止運動，你可以訓練多長時間？

無	0-5 分鐘	6-10 分鐘	11-15 分鐘	> 15 分鐘	分數
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
0	4	10	14	20	

8c. 運動時由於膝痛令你停止運動，你可以訓練多長時間？

無	0-5 分鐘	6-10 分鐘	11-15 分鐘	> 15 分鐘	分數
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
0	2	5	7	10	

APPENDIX V

OPEN ACCESS Freely available online

PLOS ONE

Shear Elastic Modulus on Patellar Tendon Captured from Supersonic Shear Imaging: Correlation with Tangent Traction Modulus Computed from Material Testing System and Test–Retest Reliability

Zhi Jie Zhang^{1,2}, Siu Ngor Fu^{1*}

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Abstract

Characterization of the elastic properties of a tendon could enhance the diagnosis and treatment of tendon injuries. The purpose of this study was to examine the correlation between the shear elastic modulus on the patellar tendon captured from a Supersonic Shear Imaging (SSI) and the tangent traction modulus computed from a Material testing system (MTS) on 8 fresh patellar pig tendons (Experiment I). Test–retest reliability of the shear elastic modulus captured from the SSI was established in Experiment II on 22 patellar tendons of 11 healthy human subjects using the SSI. Spearman Correlation coefficients for the shear elastic modulus and tangent traction modulus ranged from 0.82 to 1.00 (all $p < 0.05$) on the 8 tendons. The intra and inter-operator reliabilities were 0.98 (95% CI: 0.93–0.99) and 0.97 (95% CI: 0.93–0.98) respectively. The results from this study demonstrate that the shear elastic modulus of the patellar tendon measured by the SSI is related to the tangent traction modulus quantified by the MTS. The SSI shows good intra and inter-operator repeatability. Therefore, the present study shows that SSI can be used to assess elastic properties of a tendon.

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Introduction

Tendons are involved in every human motion and subjected to high loads. A tendon consists of parallel collagen fibers to resist elongation [1] and exhibits viscoelastic properties for force production and absorption [2]. Alteration in tendon stiffness may compromise the tendon's capacity to absorb and respond to loads [3,4]. Quantification of its elastic properties may help improve our understanding of the underlying causes of tendon-related disorders, such as tendinopathy.

The elastic properties of tendons have been determined using animal [5] and cadaveric [6] tendons undergoing ramped stretching imposed by a motor of a material testing system (MTS). It has not yet been established, however, whether findings from isolated excised tendons can be applied to *in-vivo* physiological functions [4]. Ultrasonography is a non-invasive method for measuring the elastic properties of the human tendon *in-vivo* [4,7]. This method has been used to examine changes in tendon stiffness associated with exercise [8] and

aging [9]. However, complex methodologies and long acquisition time are the drawbacks of this approach [7].

Recently, ultrasound elastography has been applied to investigate the mechanical properties of the Achilles tendon [10]. Ultrasound elastography (strain imaging) is a real-time imaging tool for the *in vivo* estimation of tissue strain distribution [11,12]. A compressive force is applied to the tissue surface inducing transverse tissue displacement, which is calculated from the echo signal set before and after the compression [13]. The force can be applied manually (freehand elastography) or mechanically (transient elastography). The absolute value of the elastic properties cannot be provided from freehand ultrasound elastography. Manual compression may alter the mechanical properties of the testing tissues.

Supersonic shear imaging (SSI) operates on a transient elastography principle. It produces elastography images based on the combination of a radiation force and an ultrafast ultrasound acquisition imaging system capable of capturing in real time, the propagation of the resulting shear waves [14]. The elastic modulus can be calculated from the velocity of the

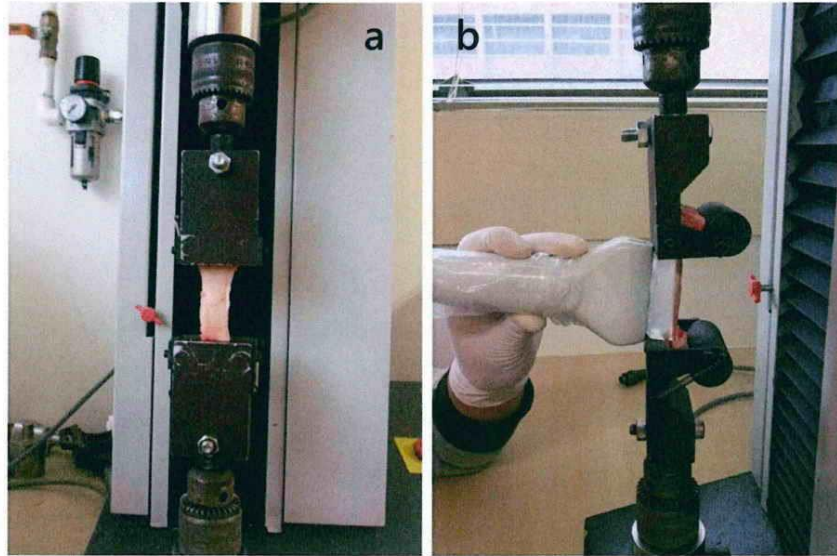


Figure 1. Experiment set-up. (a) The tendon was clamped carefully onto the MTS (anterior view); (b) The gel pad and transducer were put lightly on the tendon in order to capture its elasticity using Supersonic Shear Imaging.

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propagating wave when a faster velocity indicates a greater elastic modulus. Therefore, the elastic modulus can be calculated by measuring the propagation of shear waves. A light touch on the skin with the ultrasound probe is suggested by the manufacturer and a quantitative elasticity map can be computed from the system within a few milliseconds. The objectives of this study were: (1) to assess the correlation of the shear elastic modulus captured from an SSI and the tangent traction modulus from a MTS (Experiment I); and (2) to assess the reliability of the shear elastic modulus captured from the SSI, by using test–retest measurements on the patellar tendons of healthy subjects (Experiment II).

Experiment I

Methods

Fresh knee joints of pigs are dissected and sold in local food market. It was not necessary, therefore, to apply for ethics approval. A total of eight fresh patellar pig tendons were dissected carefully from the patella and tibia, and all soft tissues were removed from around the knee joints, leaving only

patella and small tibia tuberosity (Figure 1). The length of the specimens were measured by a plastic meter (Smartmax; SM-103) that was used to adjust the distance of the 2 clamps of a material testing system (MTS Synergie 200, MTS System Corporation, Ivry sur Seine Cedex, France) (Figure 2). The room temperature was controlled at 25 °C.

The patella and tibia of the dissected patellar tendon were connected to the 2 clamps with their fibers aligned using applied force. The tensile force (F) was applied to the 2 crossheads causing incremental displacement of the 2 clamps (d) in steps of 0.2mm at a test speed of 20mm/min until the force reached 10N. A maximum force of 10N was chosen based on our own pilot study. When the applied force exceeded 10N, the elastic modulus reached the saturation level of SSI (300kPa). The force was captured by the load cells and the displacement of the crossheads was measured by an extensometer (MTS model 634.12F-24, MTS System Corporation, Eden Prairie, MN). Both values were displayed on-line on a computer attached to the material testing system.

After the specimen was secured between the two clamps, a gel pad (ULTRA PHONIC FOCUS; Conforming gel pad; USA) was fixed onto the surface of the specimen in order to capture

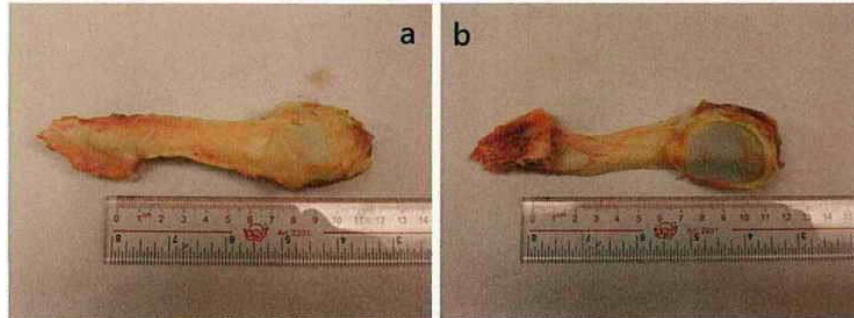


Figure 2. Photographs of a representative patellar tendon. (a) Anterior view for patellar tendon; (b) The length of the patellar tendon was measured by a meter.

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clear imaging. The cross-sectional area of the tendon was measured using the B-mode of the An Aixplorer® ultrasound unit (Supersonic Imaging, Aix-en-Provence, France) in conjunction with a linear-array transducer at 4-15 MHz frequency and a high frame rate (up to 20,000 frames/s). Once a clear image of the tendon had been achieved, the shearwave elastography mode was activated. To avoid the effect of anisotropy on the measurement, the probe was aligned with the direction of the fibres. One image was captured from each increment of 0.2mm when the tension was constant during the elastographic measurements. The image was frozen when the entire ROI was covered by the color and stored for off-line analysis.

Off-line analysis was conducted on the captured images from the SSI. A circle that delineated the region of interest (ROI) for the measurement of the elastic modulus was placed at the proximal, middle and distal parts of the SSI acquisition box on the patella tendon (Figure 3). The colours represented the stiffness of the tissues within the region of interest and ranged from red (hard) to blue (soft). The diameter of the ROI was determined by the width of the patellar tendon. Mean values of the elastic modulus on the patella tendon within the ROI were assessed by the built-in specific quantification program. The elastic modulus (E) was computed from the system based on the following equation, $E = 3\rho V_s^2$. Where density ρ is assumed to be constant (1000kg/m^3) in human soft tissue and V_s is the velocity of the shear wave propagation [14]. Due to tendon anisotropy, the shear elastic modulus was used in this study [15]. Thus, all the values obtained using the SSI was divided by 3 in this study.

Data processing was conducted using the software LabVIEW 8.6 (LabVIEW Professional Development System, USA). The loads (F) and crosshead displacement (d) were recorded during testing by a computer attached to the MTS. The tensile stress (σ) was calculated as the applied load divided by the

tendon cross-sectional area (A_0) ($\sigma = F/A_0$). The tensile strain (ϵ) was calculated as displacement divided by initial length (L_0) ($\epsilon = d/L_0$). The tangent modulus was computed by a self-written programme based on the formula $E = \Delta\sigma/\Delta\epsilon$. Spearman's rank correlation tests were used to assess the level of correlation between the shear elastic modulus of the tendon captured from the SSI system and the tangent traction modulus calculated from the MTS.

Experiment II

The subjects were fully informed of the procedures as well as the purpose of this study. Written consent was obtained from each subject. This study protocol was approved by the Human Subject Ethics Subcommittee of the Department of Rehabilitation Science, the Hong Kong Polytechnic University.

Eleven healthy subjects (8 male, 3 female; age: 26.1 ± 3.2 years, weight: 58.7 ± 12.3 kg, height: 169.2 ± 10.0 cm) were invited to participate in this study. These subjects underwent a clinical examination to exclude patellar tendon disorders, such as patellar tendinopathy. A further exclusion criterion for participation of healthy subjects was a history of knee injury or surgery. Clinical examinations, consisting of an assessment of local tenderness over the patellar tendon and pain aggravation during single leg squatting were performed by an experienced physiotherapist.

Each participant was examined while lying supine with the knee at 30° of flexion [16]. The knee was supported on a firm towel and a custom-made ankle stabilizer was used to keep the leg in neutral alignment on the coronal and transverse planes. Prior to testing the subject was allowed to have 5 minutes rest in this position, to ensure the elastic modulus of the patellar tendon was evaluated at resting status. The room temperature was controlled at 25°C .

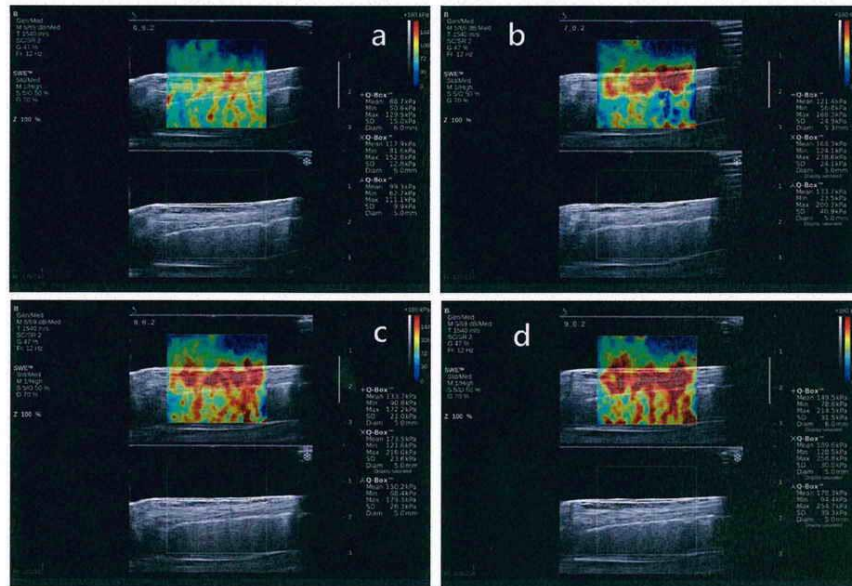


Figure 3. Changes of shear elastic modulus of pig patella tendon (PT) during increasing loading (from a to d). Upper images show the color-coded box presentation of the PT elasticity (red color represents stiffer area and green color represents softer area) with the measurement circle representing the region of interest and its corresponding shear elastic modulus demonstrating under Q-Box™ on the right. The 3 measurement circles were placed on the proximal, middle and distal part of the PT (The diameter of the measurement circle was dependent on the thickness of the tendon). The bottom images show the longitudinal grey scale sonograms of the PT to ensure the capture of clear images.

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The elastic modulus of the patellar tendon was measured using an Aixplorer® ultrasound unit (Supersonic Imaging, Aix-en-Provence, France) in conjunction with a linear-array transducer at 4–15 MHz frequency and a high frame rate (up to 20,000 frames/s). The transducer was placed longitudinally on the patellar tendon with the knee flexion of 30°. The shear wave elastography mode was then activated to measure the elastic modulus of the proximal part of the patellar tendon. The transducer was stationed on the skin, with a light pressure on top of a generous amount of coupling gel, perpendicularly on the skin's surface. The transducer was kept motionless for 8–12 seconds during the acquisition of the SSI sonogram [17]. Images were frozen when the color in the region of interest was uniform and were then stored for off-line analyses. In total, 3 images were captured for the tendon on each knee.

Two operators (I and II) participated in the inter-operator investigation. Operator I had about 5 years of experience in ultrasound scanning and SSI training. Operator II was a sports

physiotherapist with about 2 years of experience in ultrasound imaging as well as SSI short course training. The operators took turns to examine each subject's patellar tendon at one-hour intervals; and by Operator II with a 3-hour interval. The results were not communicated until all subjects had been examined.

A circle that delineated the region of interest (ROI) was centered at the proximal part of the tested tendon (Figure 4). The diameter of ROI was defined by the thickness of the tendon, which was the distance between the superior and inferior borders of the proximal part of the patellar tendon. The mean values of the elastic modulus on the patellar tendon within the ROI were computed from the system.

The dependent measure for analysis was the averaged mean tendon shear elastic modulus from all 3 images of the patellar tendon. Both intra and inter-operator reliability were examined using intraclass correlation coefficients (ICC). ICC(1,3) was used to determine the intra-operator reliability

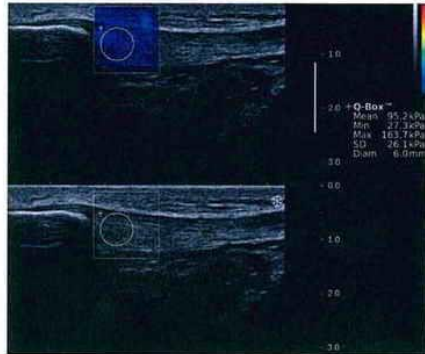


Figure 4. Typical example of elastic modulus measurement for the proximal patellar tendon on a healthy subject. Upper images show the color-coded box presentation of the PT elasticity (the red color represents the stiffer area and the green color represents the softer area) with the measurement circle representing the region of interest and its corresponding elastic modulus demonstrating under Q-Box™ on the right. The transducer was kept motionless for 8 to 12 seconds during the acquisition of the SSI sonogram and the diameter of the measurement circle was defined by the thickness of the tendon. The bottom images show the longitudinal grey scale sonograms of the PT to ensure the capture of clear images.

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and ICC (2,2) was computed to examine the inter-operator reliability [18]. The coefficient of variance (CV) was calculated [using the formula $CV = (\text{standard deviation}/\text{mean}) \times 100\%$]. The standard error measurement was computed (using the formula $SEM = \text{standard deviation} \times \sqrt{1-ICC}$), and minimal detectable difference was calculated (using the formula $MDD = 1.96 \times SEM \times \sqrt{2}$). All reliability coefficients were interpreted as follows: below 0.499 as poor, 0.500 to 0.699 as moderate, 0.700 to 0.899 as good, and 0.900 to 1.000 as excellent [19]. The statistical analysis was performed using SPSS Version 17.0 for Windows (SPSS Inc, Chicago, IL).

Results

Experiment I

Table 1 shows the cross sectional area, resting length, shear elastic modulus and tangent traction modulus obtained from the 8 fresh pig patellar tendons. Figure 5 depicts the relationships among the SSI and MTS measurements in the specimens. Significant correlations were found between the 2 variables in all tested specimens with the correlation coefficient ranging from 0.82 to 1.00 (all $p < 0.05$, Table 2 Figure 5).

MTS and SSI denote Material Testing System and Shear Supersonic Imaging.

Experiment II

The intra and inter-operator reliabilities for the measurements of the proximal patellar tendons in the eleven healthy subjects (22 patellar tendons) were excellent. The intra-operator reliability value for ICC was 0.98 and for MDD was 4.27kPa, corresponding to an SEM of 1.54kPa and CV of 29.53%. With regard to the inter-operator reliability, the ICC value was 0.97 and the MDD value was 13.68kPa, corresponding to an SEM of 1.46kPa and a CV of 25.89% (Table 3).

Discussion

Significant correlations were found between the shear elastic modulus on the patellar tendon captured from the SSI and the tangent traction modulus computed from the MTS. Excellent intra and inter-operator reliabilities were obtained when the SSI was performed on the healthy patellar tendons.

High correlations were observed between the shear elastic modulus measurement using the SSI and the tangent traction modulus obtained from the MTS of the 8 pig patellar tendons. We also observed differences in the slopes of different specimens. Hence, there was no single transformation from the elastic modulus to the tangent modulus that covered all specimens. We postulate that such differences might relate to the following factors. The MTS is known to have several inherent limitations. The stretching of the clamped fibrous tissues is associated with some slippage of inner fibres [4]. In this study, the bones (patella/ tibia tuberosity) were fixed tightly on the MTS. Some of the stretching force imposed from the MTS might have been taken up at the system-bone interface, which might have been different on each tendon. Soft tissues (fat/fascia) around the tendons had to be removed by hand. Any remaining tissues would have contributed to some of the loads. Despite great care being exercised to clear the tissue around the tendons, the amount of tissue remaining might have been different on each tendon. The tangent modulus computed from the MTS might thereby have been influenced by the above two technical issues to different extents on the tested tendons. On the other hand, intrinsic factors such as age and gender can contribute to the viscoelastic properties of the tendon. The viscoelastic properties of the patellar tendon may alter structurally with age, for example changes in collagen content. In an animal model, Haut et al. [20] reported a decrease in the collagen content of the patellar tendon with age. Similar observations were also found in human studies [21,22]. Gender may also affect the viscoelastic properties of the patellar tendon. Kubo [23] reported that the stiffness of the medial gastrocnemius tendon was significantly higher in males (approximately 37%) than in females. Note that, in this study, the elastic modulus was calculated based on the assumption that the density of the medium was a constant. This, however, may have been affected by the age or gender of the testing tendons, as we did not control for either of these factors.

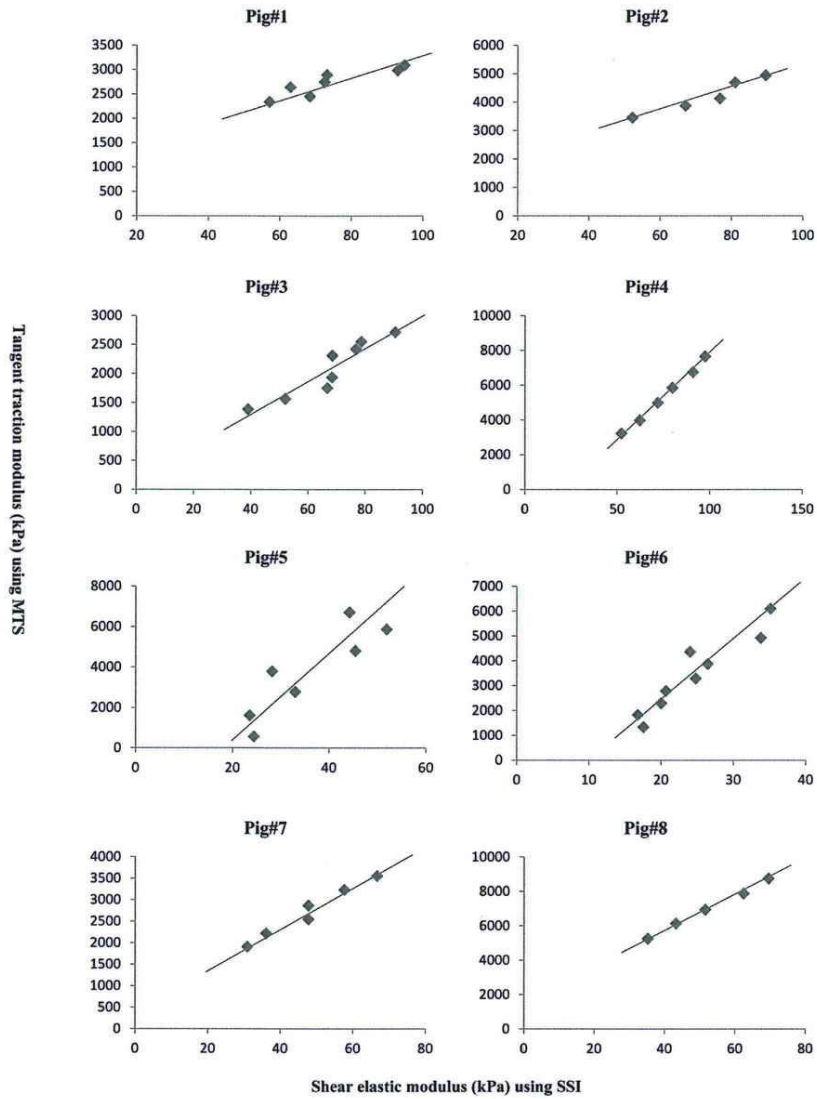


Figure 5. Correlations between shear elastic modulus of tendon captured from a Shear supersonic imaging and tangent traction modulus of tendon computed from a material testing system.
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Table 1. Cross sectional area, resting length, shear elastic modulus and tangent traction modulus obtained from fresh patellar tendon.

pig	Cross sectional	Resting length	Mean shear elastic	Mean tangent
	area (mm ²)	(mm)	modulus (kPa)	traction modulus (kPa)
1	86.3	40.0	74.6	2736.5
2	114.0	45.0	73.3	4216.5
3	95.3	42.0	67.6	2079.8
4	71.0	65.0	75.8	5410.5
5	48.6	44.0	35.8	3736.5
6	54.0	55.0	24.3	3420.4
7	48.3	50.0	47.8	2722.7
8	46.6	40.0	52.4	6989.3

Table 2. Spearman's rank correlation coefficient between the shear elastic modulus and tangent traction modulus of tendon obtained from SSI and MTS, respectively.

pig	Rho	p-value
1	0.96	0.000
2	1.00	0.000
3	1.00	0.000
4	1.00	0.000
5	0.82	0.023
6	0.93	0.000
7	0.99	0.000
8	1.00	0.000

Table 3. Intra-class coefficient (ICC) values for intra and inter-operator reliabilities on the shear elastic modulus of patellar tendon.

Intra-operator reliability (Mean±SD) (kPa)					
Test 1	Test 2	ICC	95%CI	SEM	MDD
36.9±10.9	36.0±9.5	0.98	0.93-0.99	1.54kPa	4.27kPa
Inter-operator reliability (Mean±SD) (kPa)					
Operator I	Operator II	ICC	95%CI	SEM	MDD
36.9±10.9	36.7±9.5	0.97	0.93-0.98	1.64kPa	4.54kPa

Abbreviations: ICC = Intra correlation coefficient; CI = confidence index; SEM = standard error measurement; MDD = minimal detectable difference

To the best of our knowledge, the present investigation is the first to report the relationship between the modulus elastic using the SSI and the tangent traction modulus from the MTS measurement on tendons. All of the reported studies compared the muscle elastic modulus with muscle activities based on electromyography (EMG) studies. Nordez's [24] was the first group to report that the elastic modulus of the biceps muscles of 6 healthy subjects was related strongly to the EMG activity level during muscle isometric contraction. Significant linear relationships between the elastic modulus and the individual

muscle force were also reported on the small hand muscles such as the abductor digiti minimi and first dorsal interosseus [25], indicating that the elastic modulus could be used to estimate tension inside tissues. In addition, Maisetti [27] reported a significant correlation between the elastic modulus and medial gastrocnemius muscle force during passive stretching. The correlations reported in the present study were possible only because both moduli were increased when the tension was increased.

The ultrasonography method has been used extensively over the last 20 years to assess the elastic properties of tendons. The elastic properties of the tibialis anterior and gastrocnemius tendons have been measured by ultrasound imaging as a method of estimating tendon-aponeurosis elongation during the tensile loading induced by the contraction of the in-series muscle [4]. Hansen [7] reported that the method to assess the elastic properties of human patellar tendon is reliable using ultrasonography with EMG during quadriceps muscle isometric contraction. The EMG has been used simply by some authors to correct force measurements from the antagonist contribution. Although this is a useful method to evaluate the elastic properties of the tendon, there are some limitations, such as complex techniques (transducer fixation technique), knee joint movement control, time consumption, complicated data analysis procedures and dependence on the muscle contraction [7]. Due to above the barriers, it is difficult to apply this method in the hospital and clinic to estimate the elastic properties of tendons. The SSI has overcome these limitations in the elastic properties measurement of the tendon, and this study has supported it as a relatively convenient method of measuring the elastic property of the patellar tendon.

Experiment II evaluated the intra and inter-operator reliability in obtaining the SSI elastic modulus measurements of the healthy participants' proximal patellar tendons at the rest position within-day. If subjects with tendon disorders are assessed several times by different examiners, it is important to know the intra and inter-operator reliabilities. The results of this study have demonstrated that the SSI of the proximal patellar tendon has excellent intra and inter-operator reliabilities in healthy subjects. The SSI can be used to assess disease progression and the efficacy of intervention on patellar tendons when evaluations have to be conducted at different time points.

It has been reported that the SSI is a reliable method for evaluating the elastic properties of muscles. Lacourpaille [26] reported that the ICC value of intra- and inter-operator reliability among various muscles (gastrocnemius medialis/tibialis anterior/rectus femoris/biceps brachii/ triceps brachii/ vastus lateralis) during resting status ranged from 0.81 to 0.95 and 0.42 to 0.94, respectively. Another study revealed that the intra-operator reliability of the elastic properties of biceps brachii during 3% and 7% maximal EMG activity were good (3%, ICC=0.89; 7%, ICC=0.94) [23]. Our intra and inter-reliabilities results of 0.98 and 0.97 respectively are higher than those reported from these studies of muscles. One of the reasons for this may be related to the different structures of the muscle and tendon. A tendon consists of parallel collagen fibers, and it is easier to align and re-align the US probe with

the tendon than with the muscle fibers, resulting in higher reliability.

Drakonaki et al. [10] obtained moderate to good intra and inter-operator reliability in assessing the stiffness of Achilles tendons using real-time freehand ultrasound elastography which depends on compressive force. In their study, 25 healthy subjects were recruited for the assessment of tendon stiffness at the middle third of the free tendon and the middle part between the myotendinous junction and the calcaneal insertion. The intra and inter-operator reliabilities ranged from moderate to good (0.51-0.78). One of the major differences between the SSI and real-time freehand ultrasound elastography is that the mechanical vibration is induced automatically by using a radiation force of ultrasound beams [14]. Thus, the SSI technique does not depend on external force from operators. This may be one of the main explanations why the SSI technique is more reliable than real-time free-hand ultrasound elastography.

In addition, our study calculated the MDD, which can provide a value to reflect a real change as a reference for future study. In terms of our findings, the measurement of the elastic modulus of the patellar tendon should be greater than 4.27kPa (the same operator) and 4.54kPa (different operator) to reflect real changes with retested measurements.

There are some advantages of the SSI technique when compared with other methods to evaluate the tendon elastic properties. First, it is a reliable and convenient technique to assess the elastic properties of the tendon. In the present study, the time required for scanning 2 tendons lasted for 5-8 minutes. Second, the operation of the machine can be learnt by a novice. Although Operator I (2years) and Operator II (5years) had different lengths of experience with the ultrasound scanning technique, the findings from the present study have demonstrated good intra and inter-reliabilities of the SSI measurement on the tendon elastic modulus, which indicates that the results could not have been influenced by the operator's experience. Finally, the SSI can be used to evaluate tendon elastic properties that are not affected by the presence of pain. The conventional approach based on ultrasonography and the EMG, induces an increase in the tensile force on the

tested tendon that might cause pain on a tendon with pathologies. These advantages of the SSI make it a promising clinical tool to follow disease progression and enhance the efficacy of different interventions.

In this study, the mean shear elastic modulus on the healthy patellar tendons ranged from 36.0 to 36.9kPa. The results from the present study were higher than those reported by Kot [17]. The mean shear elastic modulus on healthy patellar tendons being reported were 23 to 24 kPa. Such discrepancies might relate to the different method of defining the ROI. In Kot's study, the ROI was pre-determined (2mm, 3mm or 4mm). In the present study, we adopted the approach used by Nordez and Hug [24]. The diameter of the ROI was defined by the thickness of the tendon, which was the distance between the superior and inferior borders of the proximal part of the patellar tendon. Note that the tendon thicknesses in this study ranged from 3 to 7mm and different portions of the patellar tendon contain different percentages of collagen fibers [28]. Our approach, thereby, included the whole rather than portion of the tendon.

Conclusion

The present study has indicated that the shear elastic modulus on the patellar tendon measured from the SSI is related closely to the tangent traction modulus calculated from the MTS. The *in-vivo* measurement has illustrated excellent reliability of this tool. The SSI can be applied to evaluate the elastic properties of a healthy patellar tendon. The diagnostic role of this technique will be investigated by assessing the shear elastic modulus of normal and pathological tendons, as well as to monitor disease progression and the efficacy of intervention on individuals with tendon disorders.

Author Contributions

Conceived and designed the experiments: FSN ZJZ. Performed the experiments: ZJZ. Analyzed the data: FSN ZJZ. Contributed reagents/materials/analysis tools: FSN ZJZ. Wrote the manuscript: FSN ZJZ.

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Changes in Morphological and Elastic Properties of Patellar Tendon in Athletes with Unilateral Patellar Tendinopathy and Their Relationships with Pain and Functional Disability

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Abstract

Background: Patellar tendinopathy (PT) is one of the most common knee disorders among athletes. Changes in morphology and elasticity of the painful tendon and how these relate to the self-perceived pain and dysfunction remain unclear.

Objectives: To compare the morphology and elastic properties of patellar tendons between athlete with and without unilateral PT and to examine its association with self-perceived pain and dysfunction.

Methods: In this cross-sectional study, 33 male athletes (20 healthy and 13 with unilateral PT) were enrolled. The morphology and elastic properties of the patellar tendon were assessed by the grey and elastography mode of supersonic shear imaging (SSI) technique while the intensity of pressure pain, self-perceived pain and dysfunction were quantified with a 10-lb force to the most painful site and the Victorian Institute of Sport Assessment-patella (VISA-P) questionnaire, respectively.

Results: In athletes with unilateral PT, the painful tendons had higher shear elastic modulus (SEM) and larger tendon than the non-painful side ($p < 0.05$) or the dominant side of the healthy athletes ($p < 0.05$). Significant correlations were found between tendon SEM ratio (SEM of painful over non-painful tendon) and the intensity of pressure pain ($r = 0.62$; $p = 0.024$), VISA-P scores ($r = -0.61$; $p = 0.026$), and the sub-scores of the VISA-P scores on going down stairs, lunge, single leg hopping and squatting (r ranged from -0.63 to -0.67 ; $p < 0.05$).

Conclusions: Athletes with unilateral PT had stiffer and larger tendon on the painful side than the non-painful side and the dominant side of healthy athletes. No significant differences on the patellar tendon morphology and elastic properties were detected between the dominant and non-dominant knees of the healthy control. The ratio of the SEM of painful to non-painful sides was associated with pain and dysfunction among athletes with unilateral PT.

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Introduction

Patellar tendinopathy (PT) is a common and often chronic knee disorder among competitive athletes [1]. Its prevalence has been reported to be as high as 30% to 45% in athletes involved in jumping sports [2]. Subjects with PT are characterized with localized pain at the proximal patellar tendon associated with jumping and squatting activities that load the tendon [3]. Since the primary function of tendon is to transmit tensile loading, any change in its morphology and elastic properties may affect its function during normal activities.

Tendinopathy results in disruption and disorganization of the tendon fibers [4], along with increases in tendon thickness [3,5] and cross-sectional area (CSA) of the structure affected [6]. Based on ultrasound imaging, Cook et al. [7] observed hypochoic changes in human tendons with tendinopathy. The authors thereby recommended the use of ultrasonography in addition to clinical examination to confirm the diagnosis of PT. Alteration in the elastic properties of patellar tendon, however, have not been adequately described. In individuals with PT, the tendon was found to be more elastic in one study [8] but no difference was reported in another 2 studies [9–10] when compared with controls. In those studies, tendon stiffness was assessed using

ultrasound imaging with dynamometry. This technique measures the elastic properties of the whole tendon during ramped maximum voluntary isometric contraction. Clinically, pathological lesions in PT typically occur at about 5 mm from the apex of the patella [3,11], therefore site-specific evaluation at the pathological region might shed light on the changes on tissue elastic properties associated with tendinopathy.

Recently, strain imaging has been used to assess regional tendon elastic properties [12–13]. A compressive force is applied either manually or by the emission of low radiofrequency impulses via an ultrasound probe to the tendon surface causing tendon displacement. Tissue elasticity is graded as either soft, intermediate or hard and expressed in colour-coded images (elastogram) [12]. Based on this technique, the common extensor tendon was found softer in subjects with lateral epicondylitis [13] but harder among those with Achilles tendinopathy [12] than healthy controls. To date, regional-specific evaluation on tendon elasticity associated with tendinopathy is scarce and findings are conflicting. In addition, the strain imaging technique provides qualitative but not quantitative information of tissue elasticity [14–15]. Thus, the magnitude of changes could not be quantified.

The supersonic shear imaging (SSI) technique provides quantitative values of tendon elastic properties at a selected area of interest [16–20]. It relies on measuring the speed of propagation of shear waves generated by acoustic radiation force and to estimate the shear elastic modulus of soft tissues [20]. Our recent findings indicated that the patellar tendon shear elastic modulus measured using SSI has good intra- and inter-rater reliability and is correlated with the Young's modulus of the tissue [17]. Based on this technique, decreases in tendon elastic modulus in acute ruptured Achilles tendon in human subjects [18] and in partial tendon tears in porcine model [19] were reported. These studies provide the evidence base on the feasibility of using SSI for measuring tendon elastic properties. However, these findings cannot be generalized to tendon with tendinopathy because this condition is a degenerative process [21]. In addition to interruption of tendon fibrils [22], changes in fiber type [23] and increases in collagen cross-link concentration [24] were detected in tendon with tendinopathy. Based on the SSI technique, quantitative regional-specific tendon elasticity could be measured and compared between subjects with and without patellar tendinopathy. Such information could increase our understanding in regional changes on tissue elastic properties as well as the magnitude of changes.

There is also a question of how the changes in tendon morphological and/or elastic properties in individuals with PT relate to their perceived pain and dysfunctions. Increased tendon thickness has been reported to be associated with greater pain among athletes with PT [25]. To date, the relationship between elastic properties of tendon and self-perceived pain in individuals with PT has not been investigated. In view that pain and decrease in functional strength in the tendons could mean an end to the athletic career of a sportsman, it is therefore important to find out how changes in tendon morphology and mechanical properties are related to the disability and dysfunctions in people with PT, so that appropriate remedial measures can be developed.

The objectives of this study were to 1) compare the elastic modulus of patellar tendon between dominant and non-dominant sides among healthy subjects; 2) compare tendon on the painful and non-painful side of the same subject and also with healthy control subjects and; 3) determine whether changes in tendon shear elastic modulus were related to pain and dysfunction.

Materials and Methods

Ethics statement

This study was approved by the Human Subject Ethics Subcommittee of the administrating institution. The experimental procedures were conducted in accordance with the Declaration of Helsinki. The procedures of the study were fully explained to the participants and they provided their informed written consent before testing.

Study population

Subjects were recruited from the volleyball, basketball and handball teams of local universities and the community. Only males were recruited, because PT is more prevalent in male than female athletes [7]. The inclusion criteria were as follows: 1) between 18 and 35 years of age; 2) had unilateral pain in the inferior pole of patella or the proximal part of patellar tendon; 3) pain duration ≥ 3 months; 4) maximum intensity of pain in the previous week ≥ 3 using a visual analogue scale (VAS) with 0 as no pain and 10 as the worst pain; 5) VISA score ≤ 80 [26]; 6) no history of corticosteroid injection or surgery to the lower limb. All recruited subjects were physically assessed by an experienced physical therapist (WCL) who has 15 years of clinical experience and then ultrasonography examination was conducted by another physical therapist (JZ) who has 3 years of experience in ultrasound scanning. The subject was diagnosed as having PT based on the clinical examination and ultrasonography findings that included the following: 1) local tenderness in the inferior pole of patella, or the proximal part of patellar tendon; 2) pain aggravation during single leg squatting and jumping [27]; 3) thickening of proximal part of patellar tendon with area of hypochoic signals [6]. Twenty healthy athletes, with similar age and training hours but without clinical symptoms or abnormal ultrasound-based images of the patellar tendon, were recruited as the controls for this study.

All subjects filled in a form recording their age, weight, height and training duration per week. Subjects with PT completed the Victorian Institute of Sport Assessment-patella (VISA-P) questionnaire. The leg of dominance was determined by asking the subject to kick a ball [28].

Ultrasound Examination

An Aixplorer ultrasound unit (Supersonic Imaging, Aix-en-Provence, France) in conjunction with a 50-mm linear-array transducer at 4–15 MHz frequency was used in this study [20]. B-mode was used to measure the tendon thickness and CSA. Shearwave mode was used to measure the shear elastic modulus of the patellar tendon at its proximal part. This location was selected because the pathological changes with diagnostic imaging for PT are most commonly found in the proximal part of the patella at about 5 mm distal to the apex of the patella [6]. The musculoskeletal acquisition mode was used to measure the elastic modulus of patella tendon with the temporal averaging (persistence) and spatial smoothing set to medium and 6, respectively. The elastic modulus measurements were taken at 1 Hz.

Each participant was examined in supine lying with 30° of knee flexion [29]. The knee was supported on a firm towel and a custom-made ankle stabilizer to keep the leg in neutral alignment on the coronal and transverse planes. Prior to testing, the subject was allowed to have 5 minutes of rest in a comfortable position in order to unload the tension on the patellar tendon [13]. The room temperature was controlled at 25°C.

Measurement of patellar tendon thickness and CSA

The thickness and CSA in the inferior pole of patella were measured by grey scale mode (B-mode) of the Aixplorer ultrasound unit. The inferior pole of patella was identified by palpation of the examiner. The transducer was lightly located at the inferior pole of the patella and the transducer was placed longitudinally on the patellar tendon. B-mode was activated to capture the image of the patellar tendon and stored for off-line measurements. The transducer was then turned by 90° so that a transverse view of the proximal insertion of the patellar tendon could be captured and stored for off-line analysis. Three images were obtained for measuring the thickness and CSA [30]. Both knees were evaluated for all the subjects.

After obtaining the ultrasound images, off-line measurements were performed. The tendon thickness was measured using the distance measurement software in the ultrasound machine. Measurements were taken from the inferior pole of patella vertically to the superior border of the patella tendon (Fig. 1A) using a trackball. The CSA of the patellar tendon (Fig. 1B) was measured by the tracing measurement software which allows the examiner to trace the outer margin of the tendon through the trackball, and the tracing measurement software was used to calculate the total area traced. The mean from the 3 measurements were used for statistical analysis.

Eleven healthy sedentary subjects were assessed twice with one week apart for test-retest reliability of patellar tendon thickness and CSA measurements. The inter-rater coefficient of correlation of tendon thickness and CSA were 0.94 (CV = 18.75%) and 0.98 (CV = 32.30%), respectively.

Measurement of patellar tendon elastic modulus

B-mode was used to locate and align the patellar tendon longitudinally with the transducer. When a clear image of the patellar tendon was captured, the shear wave elastography mode was then activated. The transducer was stationed on the skin with light pressure on top of a generous amount of coupling gel, perpendicularly on the surface of the skin. The transducer was kept stationary for 8–12 seconds during the acquisition of the SSI map [16]. A total of 3 images were captured for the tendon on each knee for off-line analysis.

Off-line analysis was conducted and the procedures have been described in our recent paper [16]. The region of interest (ROI) was first defined by a rectangular box of 13.5 mm × 12.5 mm (biggest size provided from the manufacturer) distal to the apex of the patella and with the patellar tendon located within its centre part. In the painful tendon, the circular quantification box (Q-Box) was centered where hypoechogenicity, disruption or fragmentation

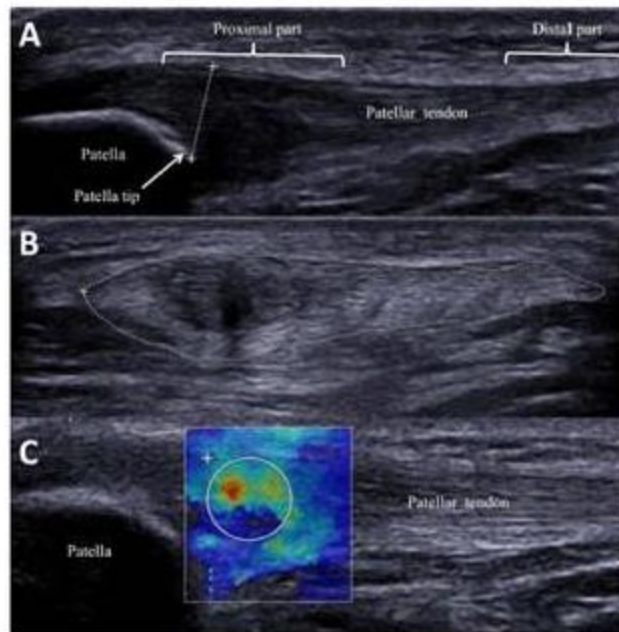


Figure 1. Sonography images of the patellar tendon (A) Thickness of the patellar tendon (dotted line) was measured from the superior border of the patellar tendon to the tip of the patella. (B) Cross-sectional area of the patellar tendon was measured by tracing the outer margin of the patellar tendon (dotted circle) (C) Shear elastic modulus of the patellar tendon was quantified by the elastography. The white circle delineates the area of interest.

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Table 1. Demography comparison between athletes with and without unilateral PT.

Variables	Control group	PT group	P Value
	(n = 20)	(n = 13)	
Age, y	24.9±4.4	22.9±4.6	0.222
Weight, kg	73.4±7.9	76.2±6.3	0.280
Height, cm	181.7±6.0	180.0±5.7	0.425
BM, kg/m ²	22.2±2.1	23.6±2.4	0.094
Sport-specific training, h/wk	7.5±3.7	5.4±2.5	0.102
Injury duration, y		1.7±1.6	
Dominant/non-dominant side (painful side)		8/5	

Values shown as mean± standard deviation; PT = patellar tendinopathy; BM = body mass index.
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of collagen fiber, or focal sonolucent were observed. Similar to a previous study [11], the pathological lesions in our subjects were detected an average of 4.6 mm (ranged from 3–7 mm) distal to the apex of the patella (Fig.1C). The diameter of the Q-Box was determined by the width of the tendon. In the non-painful tendon, the Q-Box was centered at the proximal part of the patellar tendon with consistent images and at about 5 mm from the apex of the patella. Young's modulus (E) was estimated by the SSI system based on the following equation, $E = 3\rho c^2$, where ρ is the density (constant and equal to 1000 kg/m³) and c is the velocity of the shear wave propagation based on the assumption that the tissue is isotropic. A higher Young's modulus indicates greater stiffness [20]. The mean and maximum values of Young's modulus within the Q-Box were computed and displayed in kPa at the right bottom corner of the computer screen. The mean tendon shear elastic modulus was calculated by dividing the Young's modulus generated from the system by 3 [31]. The SSI has excellent test-retest reliability on patellar tendon shear elastic modulus (ICC: 0.98; coefficient of variation: 29.53%) [17].

The ratio of mean shear elastic modulus, tendon thickness and CSA between the painful and non-painful knee was calculated as elastic ratio, thickness ratio and CSA ratio in athletes with unilateral PT.

Clinical Evaluation

Pressure pain was measured by a hand-held algometer (manufactured by pdt, Rome, Italy). Pain was provoked through a rubber disc at the end of the algometer. The participant was positioned in supine lying with 30° of knee flexion on the couch. The most painful area on the proximal patellar tendon was determined by palpation and then a 10lb force was applied via the algometer onto this area. The intensity of pain being provoked was

reported using a visual analogue scale (VAS) from 0 to 10, with 0 indicating no pain, and 10 indicating the worst pain during testing. The VAS scale is a reliable and valid scale in the evaluation of patients with anterior knee pain [32].

The VISA-P questionnaire is used to assess the severity of symptoms and functional ability in subjects with PT [33]. The questionnaire comprises 8 questions with 4 on self-perceived pain associated with a functional activity, 2 on the ability in performing functional activities, and 2 on the ability to play sport. Self-perceived pain or abilities are rated on a 10-point Likert scale with 0 being the worst pain or lowest ability and 10 as the least pain and highest ability. The total score of the questionnaire is 100 and the final score would quantify the functional level. The VISA-P questionnaire has been used for studies on PT in various athletic populations [2,34].

Statistical Analysis

Independent *t* tests were performed to compare the demographic data between athletes with and without PT. After the normality distributions were confirmed using the Shapiro-Wilk tests, paired *t*-tests were used to compare the outcome measures (thickness, CSA and shear elastic modulus of patellar tendon) between the dominant and non-dominant sides in the healthy athletes, and also the painful and non-painful sides in athletes with unilateral PT. Univariate analysis of covariance tests were used to compare between the affected side in athletes with PT and the dominant side of the controls with demographic factors that demonstrated significant group difference as covariates. Spearman's rank correlation tests were used to examine the thickness ratio, CSA ratio and elastic ratio with the pressure pain, individual and total scores of the VISA-P questionnaire. SPSS version 17.0 (SPSS Inc, Chicago, IL) was used to perform statistical analyses. A

Table 2. Comparisons of the shear elastic modulus, thickness and CSA between painful and non-painful sides in athletes with unilateral PT.

Variables	Painful side	Non-painful side	P Value
	(n = 13)	(n = 13)	
Shear elastic modulus, kPa	43.6±17.9	25.8±10.6	0.008*
Thickness, mm	6.9±1.8	4.6±0.6	0.001*
CSA, cm ²	1.7±0.4	1.4±0.3	0.002*

Values shown as mean± standard deviation; PT = patellar tendinopathy; CSA = cross sectional area. *P<0.05.
doi:10.1371/journal.pone.0108337.t002

Table 3. Side-to-side comparisons of the shear elastic modulus, thickness and CSA in healthy athletes.

Variables	Dominant side	Non-dominant side	P Value
	n = 20	n = 20	
Shear elastic modulus, kPa	27.5 ± 11.3	27.9 ± 8.4	0.968
Thickness, mm	5.6 ± 1.2	5.3 ± 1.0	0.095
CSA, cm ²	1.4 ± 0.3	1.4 ± 0.3	0.917

Values shown as mean ± standard deviation; CSA = cross sectional area.
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p value of <0.05 was considered as significant for each of the measurements.

Results

Participant's demographic data

Participants' age, height, weight, BMI and training intensity in the two groups are shown in Table 1. No significant differences were found between the two groups in age, height, weight, BMI and training intensity ($p > 0.05$), but a medium trend was found in BMI between the 2 groups ($p = 0.094$; Cohen's $d = 0.52$).

Side-to-side differences on thickness, CSA and shear elastic modulus

In athletes with unilateral PT, B-mode ultrasound measurements revealed significant differences between the painful and non-painful sides in patellar tendon thickness ($p = 0.001$) and CSA ($p = 0.002$) (Table 2). On average, the painful tendons were thickened by 33.3% and enlarged by 12.5% in CSA than the non-painful side. The shear elastic modulus in the painful side (mean 43.6 kPa) was significantly higher than the non-painful side (mean 25.8 kPa) by 40.8% ($p = 0.008$).

Side-to-side differences on the outcome measures were not observed in healthy athletes. There were no significant differences on the patellar tendon morphology and elastic properties between the dominant and non-dominant sides ($p > 0.05$) despite a trend of increase in patellar tendon thickness in the dominant than the non-dominant leg ($p = 0.095$) (Table 3). The mean tendon thickness, CSA and shear elastic modulus were 5.6 mm, 1.4 cm² and 27.5 kPa in the dominant leg; 5.3 mm, 1.4 cm², and 27.9 kPa in the non-dominant leg.

Comparison of shear elastic modulus, thickness and CSA between healthy athletes and athletes with unilateral PT

Group differences were observed on the patellar tendon morphology and elastic properties between athletes with PT and

without PT. The mean group differences on the patellar tendon thickness and CSA were 1.3 mm and 0.3 cm², respectively (Table 4). The shear elastic modulus was increased from 27.5 kPa to 43.6 kPa (by 56.9%, $p = 0.003$) in the painful tendon among athletes with PT when compared with the controls.

Relationships between changes in tendon morphology, elastic properties, pressure pain and dysfunctions

Table 5 shows the relationships between changes in tendon properties, pressure pain and dysfunctions. Significant negative correlation was found between elastic ratio and VISA-P scores ($r_{ho} = -0.61$; $p = 0.026$) (Fig. 2A). Significant positive correlation was found between elastic ratio and pressure pain ($r_{ho} = 0.62$, $p = 0.024$) (Fig. 2B) and negative relationships were established between elastic ratio and self-perceived pain based on the sub-scores from the VISA-P questionnaire (r_{ho} ranged from -0.63 to -0.67 ; $p < 0.05$) (Fig. 2C, 2D, 2E, 2G, 2H) except for the knee extension ($r_{ho} = -0.26$, $p = 0.394$) (Fig. 2F). A higher ratio, greater differences between the painful and non-painful tendon, was associated with greater intensity of pain with pressure and when performing forward lunge, going down stairs and single leg hopping as well as greater dysfunctions. Similar relationships could not be detected with thickness ratio and CSA ratio.

Discussion

The present study revealed changes in patellar tendon morphology and elastic properties in athletes with unilateral PT lasting from 3 months to 6 years. The painful tendons were thicker and larger in size with an increase in stiffness when compared with the non-painful side and healthy control subjects. This study also established associations between elastic properties of patellar tendon and intensity of pressure- and activity-related pain, as well as dysfunctions in basketball and volleyball players with unilateral PT.

Morphological changes such as thickening and larger CSA in pathological tendons, have been reported [3,5,6] and the changes

Table 4. Comparisons of the shear elastic modulus, thickness and CSA of the patellar tendon on the painful side of athletes with unilateral PT and dominant side of the healthy athletes.

Variables	Control group	PT group	P Value
	n = 20	n = 13	
Shear elastic modulus, kPa	27.5 ± 11.3	43.6 ± 17.9	0.003*
Thickness, mm	5.6 ± 1.2	6.9 ± 1.8	0.019*
CSA, cm ²	1.4 ± 0.3	1.7 ± 0.4	0.032*

Values shown as mean ± standard deviation; PT = patellar tendinopathy; CSA = cross sectional area. * $p < 0.05$.
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Table 5. Spearman's rank correlations between the ratio of tendon thickness, CSA and shear elastic modulus of the painful and non-painful side with intensity of pressure pain, individuals and total VISA-P scores.

Pain	Morphology		Elastic properties
	Thickness ratio	CSA ratio	Elastic ratio
Pressure pain	0.53	-0.04	0.62*
Dorsiflexion	-0.28	-0.22	-0.65*
Knee extension	0.02	0.42	-0.28
Single leg hopping	-0.30	-0.13	-0.64*
Lunge	-0.11	-0.07	-0.63*
Ability			
Prolong sitting	-0.46	-0.15	-0.63*
Squatting	-0.14	-0.09	-0.64*
Dysfunction			
VISA-p score	-0.25	-0.07	-0.61*

Abbreviations: CSA = cross sectional area; VISA-P = Victorian Institute of Sports Assessment-patella Questionnaire; * $P < 0.05$.
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were about 22.7% in thickness [5] and 15.8% in CSA [6] in the proximal part of the patellar tendon. Cook et al. [7] even advocates the use of ultrasound imaging together with clinical examination in making diagnosis of tendinopathy. In this study, patients were confirmed to have PT based on both clinical examination and ultrasound imaging, it is therefore not surprising to find differences on the size and CSA of the pathological tendons from the unaffected side or the patellar tendon of the healthy controls. In the present study, the tendon size was increased by 35.7% and the CSA was enlarged by 21.4% in the pathological tendons. These changes could be explained by an increase in ground substance [35], collagen fiber disorganization [4], and hypercellularity [36].

One of the main findings from this study was the change in tendon elastic properties in athletes with unilateral PT using supersonic shear imaging technique. The painful tendons were stiffer than the non-painful side as well as the dominant side of the healthy controls. The measurements were made on the proximal portion of the tendon where pain was elicited on palpation and perceived during functional activities of the lower limb. Patellar tendon elastic properties were previously quantified by ultrasonography with dynamometry. Kongsgaard et al. [9] did not find any difference in the tendon stiffness between 9 healthy individuals and 8 subjects with patellar tendinopathy. A later study by Couppe et al. [10] also reported no difference on the tendon stiffness between the painful and non-painful sides of 7 badminton players with unilateral PT and also compared with 9 control subjects. Based on a relatively larger sample, Helland et al. [8] found significantly lower tendon stiffness (by 21.4%) in the patellar tendon in 13 volleyball players with PT when compared with 15 control. In these studies, elastic properties of the whole tendon were measured so as to unveil the entire physical properties of the tendon. We were more interested to investigate regional-specific changes around the pathological region, where pain is normally elicited.

Similar to our study, regional-specific increase in tendon stiffness was reported in subjects with chronic pain in the Achilles tendons by Scoufrenza et al. [12]. The stiffness of Achilles tendons was assessed by ultrasound sonoelastography at the myotendinous junction, tendon body and calcaneal entheses. Loss of elasticity was detected in the tendon body but not in the myotendinous junction

or calcaneal entheses when compared with control. On the contrary, De Zordo reported decrease in tendon stiffness at the common extensor origin in patients with lateral epicondylitis [13]. More study is required to examine whether tendinopathy associated changes in elastic properties would be different in the lower and upper limb tendons.

The study from Scoufrenza et al. [12] observed lower elastic values in areas with fragmentation and loss of fibrillar texture. In our study, the tendon shear elastic modulus was measured at the proximal part of the patellar tendon in area of hypochoic or fragmentation signals based on ultrasound imaging. On the contrary, lower tendon stiffness was detected in subjects with acute Achilles tendon rupture [18] and in partial tendon tears in a porcine model [19]. Chen et al. [18] commented that the shearwave images could not be registered in areas with hematoma. The elastic value was dramatically reduced to 0 thus lowering the mean value. Furthermore, changes that occur in tendons with tendinopathy but not in tendons with acute rupture include transition of collagen fibers between Type I and Type III [23,37]; disorganization of collagen fibers [4], increase in collagen cross-links [24] and formation of scar tissues [38]. These changes would likely increase tissue elastic modulus. Biochemical and histological tests are suggested to assess changes on collagen fibers, extracellular matrix and tendon elastic modulus.

The patellar tendon is short and thick where considerable force is transmitted. An inextensible tendon would have the most efficient force transmission. This tendon also serves other important functions such as energy storage/release upon loading and unloading and protection from muscle fiber injury [39]. To serve these functions, the patellar tendon exhibits spring-like characteristic, due to the presence of elastic components. Increased tendon stiffness in athletes with PT might be suitable for rapid and effective force transmission but could affect its function as mechanical buffer and elastic saving for economy of motion.

Besides measuring tendon elastic properties at rest, we also correlated the resting tendon elastic properties with pain and dysfunctions. Significant correlations were found between pain and dysfunctions with modulation on the tendon elastic properties but not with tendon morphology (thickness and CSA). Patients with PT are characterized with pain at the proximal patellar

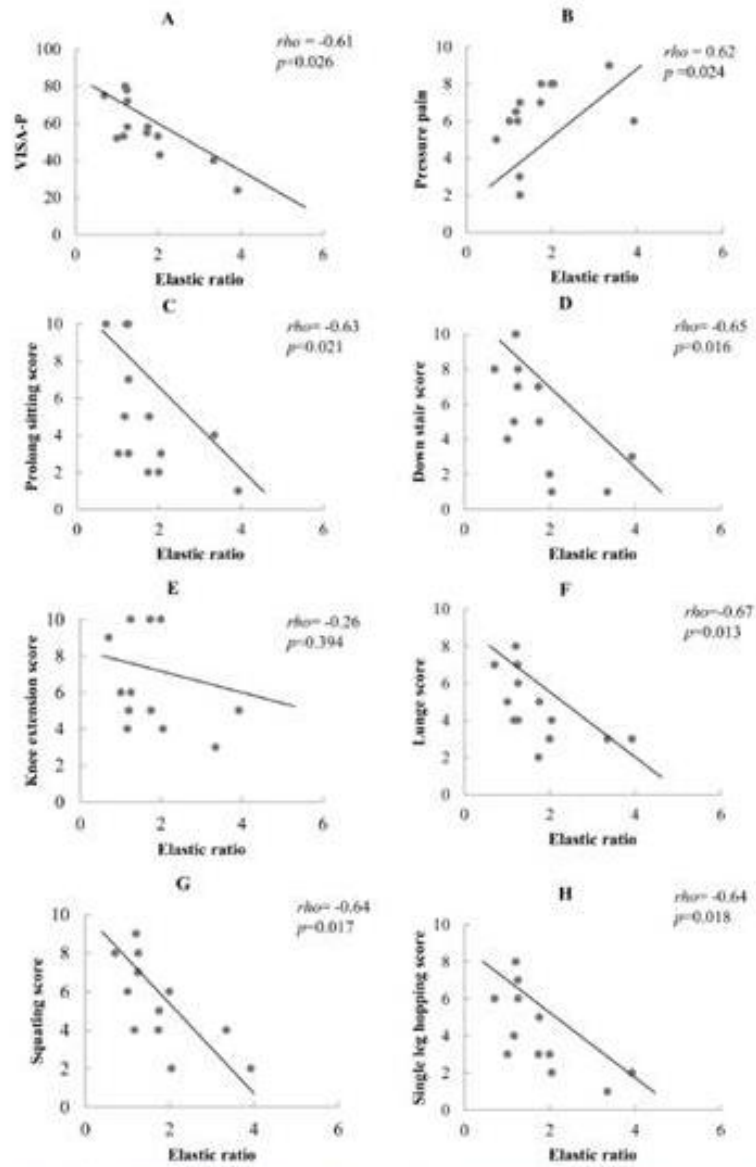


Figure 2. Correlations between elastic ratio and clinical variables. P Pressure pain, individual and total score from VISA-P, Victorian Institute of Sport Assessment-Patella. doi:10.1371/journal.pone.0108337.g002

tendon associated with activities that load the tendon [3]. The present findings provide evidence that a greater increase in stiffness of the painful tendon, i.e. greater ratio on the shear elastic modulus between the painful and non-painful side, are associated with the self-perceived pain with local pressure and during tendon-loading activities. Most importantly, the dysfunctions as reflected from the VISA-P scores are related with the modulation in the tendon elastic properties. Tendon pain is closely linked to loading while excessive energy storage and release (over stretch) to the tendon would most commonly provoke pain [40]. We could not establish a relationship between the change in tendon morphology with pain or dysfunction. Malliaras et al. [25] found that hypochoic area and diffuse thickening in patellar tendon were more likely to be painful. However, Warden et al. [41] and the present study could not establish a relationship between the CSA of patellar tendon and the VISA-P scores in athletes with unilateral PT.

Interestingly, there was not a significant side-to-side difference in the shear elastic modulus, thickness and CSA of patellar tendon between the dominant and non-dominant sides among healthy athletes. Such observations are partially in agreement with the findings from Couppé et al. [42]. The authors found similar shear elastic modulus between the dominant and non-dominant legs. The thickness and CSA of patellar tendon, however, were found to be significantly thicker and larger in the leading leg in fencers and badminton players when compared with the other leg. A proposed explanation for this finding is the unilateral/asymmetrical training in these athletes. In our study, we recruited athletes in volleyball, basketball and handball that are generally regarded as bilateral sports.

Limitations of the present study

The prevalence of PT is reported to be between 30% and 45% in the jumping athletes [2], but many of them suffered from bilateral PT. During the study period, 15 subjects with unilateral PT were recruited. Despite this small sample size, statistical significant difference was established on patellar tendon shear elastic modulus and thickness between athletes with and without PT. We also detected correlations between the tendon elastic modulus and the intensity of pain and functional scores. Such

findings illustrated influence of tendon stiffness on pain and function. However, further study with larger number of subjects and in female athletes are suggested to support the present findings. Also, we conducted evaluation of tendon elastic properties on the proximal patellar tendon that does not represent the entire tendon. This is valid because the pathological changes in patellar tendon occurred mostly in the inferior pole of patella or proximal part of the tendon [6]. However, further study measuring tendon elastic modulus at different portions of the tendon would provide information on whether changes are isolated at the pathological region. In addition, ultrasound imaging was used to determine pathological lesions that had not been verified with histological tests. The present study was a cross-sectional study, we could not determine whether a stiffer proximal patellar tendon was the cause or consequence of the PT. Finally, only male athletes were recruited in this study, thus the findings from this study may not be generalized to female basketball, handball and volleyball players.

Conclusions

The present study revealed changes in both morphology and elastic properties at the painful part of patellar tendon in athletes with unilateral PT. The affected tendons are stiffer, thicker and have larger cross-sectional area than the non-painful side and the tendon of healthy controls. In addition, the ratio of the painful and non-painful tendon elastic properties is associated with the intensity of pressure pain and VISA-p scores in athletes with unilateral PT.

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Author Contributions

Conceived and designed the experiments: SNF. Performed the experiments: ZJZ, WCL. Analyzed the data: SNF, ZJZ. Contributed reagents/materials/analysis tools: SNF, GYFN. Wrote the paper: ZJZ, SNF, GYFN. Manuscript editing: GYFN, SNF.

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Elastic Modulus of Muscle and Tendon with Shear Wave Ultrasound Elastography: Variations with Different Technical Settings

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Abstract

Standardization on Shear wave ultrasound elastography (SWUE) technical settings will not only ensure that the results are accurate, but also detect any differences over time that may be attributed to true physiological changes. The present study evaluated the variations of elastic modulus of muscle and tendon using SWUE when different technical settings were altered. The results of this study indicated that variations of elastic modulus of muscle and tendon were found when different transducer's pressure and region of interest (ROI)'s size were applied. No significant differences in elastic modulus of the rectus femoris muscle and patellar tendon were found with different acquisition times of the SWUE sonogram. The SWUE on the muscle and tendon should be performed with the lightest transducer's pressure, a shorter acquisition time for the SWUE sonogram, while measuring the mean elastic modulus regardless the ROI's size.

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Introduction

The biomechanical properties of the musculoskeletal system are difficult to assess because these structures consist of complex active or passive tissues [1]. When an electrical excitation occurs in the muscle fibers, a mechanical response will result, in the form of shortening, in addition to a modification of the mechanical properties, in the form of hardening. Understanding muscle mechanical properties is essential in clinical diagnosis and research on musculoskeletal injuries and movement-related disorders, and the application of this knowledge to patient care is central to rehabilitation [2].

Stiffness of soft tissues is clinically assessed with functional examinations (e.g., the manual muscle test), validated clinical scales (e.g., modified Ashworth scale), force measurements using hand-held and isokinetic dynamometers, and surface and fine wire electromyography (EMG). While these measurements provide information that clinicians can use to track the changes in muscle function in their patients, some are subjective or unreliable [3,4].

Recently, imaging techniques have been applied to investigate muscle function. Extensive research in the field of muscle activity measurements by MRI underscores the important role of muscle MR in neurophysiologic research, diagnosis, and therapy [5–7]. Magnetic resonance elastography (MRE) is a non-invasive elasticity imaging technique based on a phase-sensitive MR sequence that detects the propagation of shear waves generated by an external vibrator. Its applications cover several fields, including orthopedics, sports medicine, physical medicine and rehabilitation, endocrinology, and rheumatology; these mainly

help to investigate the effects of treatment for patients with muscle spasticity by using quantitative analysis. However, the spatial resolution of this technique remains limited and the large size of mechanical vibrator constrains measurement conditions, which limits the application of MRE to the musculoskeletal system.

Shear wave ultrasound elastography (SWUE) is a new real-time diagnostic imaging technique with freedom capabilities that uses ultrasound to quantitatively assess tissue differences in stiffness. SWUE operates on transient elastography principles, by measuring the propagation of shear waves induced by vibrations applied at various frequencies, which are then used to estimate the elastic modulus of the relaxed and contracted quadriceps muscle group [8]. Subsequently, transient elastography was applied to muscles to quantify the anisotropic properties of muscle tissue [1].

Although SWUE has an acceptable reliability on the assessment of muscle and tendon [9], information regarding the technical aspect on the SWUE is still scarce, with a lack of consensus. The tissue compression might still be operator dependent, and even a slight touch of the skin might influence the elastography result [10]. Few studies on SWUE have reported the choice of the region of interest (ROI)'s size [1,8], while its effect on SWUE measurements has not yet been investigated. SWUE enabled the computation of a quantitative elasticity map of an organ in just a few milliseconds [11], which differed from the 8–12 second acquisition time of the SWUE sonogram, suggested by manufacturer's preferred setting.

Standardization on SWUE technical settings will not only ensure that the results are accurate, but also detect any differences over time that may be attributed to true physiological changes.

The present study aimed to evaluate the variations of elastic modulus of muscle and tendon using shear wave ultrasound imaging when different technical aspects were altered: transducer's pressure, ROI's size, and acquisition time.

Methods

Ethics Statement

Informed, written consent was obtained from each subject. This study was approved by the Human Subject Ethics Subcommittee of the Department of Rehabilitation Sciences, the Hong Kong Polytechnic University.

Subjects

Twenty healthy subjects (14 males, 6 females) were recruited in this study. Thigh muscles of 40 legs from 20 subjects were assessed by SWUE. Exclusion criteria for the healthy subjects included a clinical history of any knee pain/injuries. The age range of the subjects was 21–33 y (mean, 26.4±3.5).

Equipment

All ultrasound examinations were performed with the Aplio[®] ultrasound unit in conjunction with a 4 to 15 MHz, 40-mm linear transducer (Supersonic Imaging, Aix-en-Provence, France).

Muscle and Tendon Ultrasound Examination

All ultrasound examinations were performed by the same operator (BK) and the operator was blinded to elastic modulus measurements obtained during the scanning. In the ultrasound examination, subjects laid supine on the examination couch in a room set at 25°C, with the knee at 30° of flexion [12]. A custom-made ankle stabilizer was used to maintain the hip in neutral alignment. The subject laid in this position for five minutes before the commencement of the examination, to ensure the elastic modulus of muscle and tendon were measured at resting phase. The left and right rectus femoris (RF) and patella tendon (PT) were assessed separately. The scanning site for RF ultrasound was determined with reference to the recommended placement of surface electrodes for electromyography (EMG) [13].

Both the RF and PT were initially identified by conventional grayscale ultrasound. Transverse view of the RF was obtained by placing the transducer on the marked point. Once the muscle fibers were identified, the transducer was oriented 90° to obtain the longitudinal view. Longitudinal scan plane of the PT was performed by placing the transducer at the level of the distal patella, with the knee at 30° of flexion to straighten the tendon, avoiding anisotropy. SWUE mode was then activated to measure the elastic modulus of muscle and tendon. The transducer was stationed very lightly on top of a generous amount of coupling gel, perpendicularly on the surface of the skin. The transducer was kept motionless for 8 to 12 seconds during the acquisition of the SWUE sonogram.

A circle that delineated the region of interest (ROI) for the measurement of elastic modulus was placed at the center of the SWUE acquisition box on both the RF and PT. The diameter of the ROI was defined by the thickness of the RF and PT [14]. Maximum and mean values of Elastic modulus (in kPa) on the RF and PT within the ROI were estimated by the built-in specific quantification program.

Transducer's Pressure

The transducer's pressure was categorized into 3 levels: light, moderate and hard. Light pressure was defined as placing the transducer very lightly on top of a generous amount of coupling

gel on the surface of the skin without further applying artefactual areas of stiffness to the RF and PT. Hard pressure was presented by placing the transducer with a great force that could deform the thickness of the RF and PT, where moderate pressure was presented by placing the transducer with a gentle force that could just barely deform the thickness of the RF (Figure 1) and PT (Figure 2). When evaluating the difference on the elastic modulus of the RF and PT with different transducer pressures applied, the other two technical parameters were fixed at the preferred/suggested settings according to the manufacturer.

ROI's Size

ROI's size was categorized into three levels (8, 10, and 12 mm diameter) for the RF (Figure 3) and three levels (2, 5, and 4 mm diameter) for the PT (Figure 4), respectively. Various sizes of ROI were placed in a concentric manner in the center of the SWUE acquisition box on both the RF and PT. When evaluating the difference on the elastic modulus of the RF and PT with ROI's size used, the other two technical parameters were fixed with the preferred/suggested settings of the manufacturer.

Acquisition Time of the SWUE Sonogram

Acquisition time of the SWUE sonogram was categorized into 4 levels: 5, 10, 15, and 20 seconds. The transducer was kept motionless for 5, 10, 15, and 20 seconds during the acquisition of the SWUE sonogram, and the corresponding elastic modulus of the RF and PT was estimated respectively. When evaluating the difference on the elastic modulus of the RF and PT with different acquisition times of the SWUE sonogram, the other two technical parameters were fixed with the preferred/suggested settings of the manufacturer.

Statistical Analysis

The level of significance of the difference between the groups in the three investigated technical parameters (Transducer's pressure, ROI's size, Acquisition time of the SWUE sonogram) in the maximum and mean values of the elastic modulus of the RF and PT were calculated by the Friedman Test with Dunn's multiple comparison tests for post-hoc analysis. GraphPad InStat software was used for all statistical analyses (GraphPad Software Inc., San Diego, CA, USA).

Results

A total of 40 RF and PT were evaluated with ultrasonography in the 20 healthy subjects. Results showed that there were significant differences ($p < 0.05$, Table 1) in both the maximum and mean value of the elastic modulus of the RF and PT when different transducer's pressure was applied. Further evaluation using post-hoc Dunn's multiple comparison tests indicated that light pressure gives a significantly smaller maximum and mean elastic modulus than when measuring with moderate and hard pressure, while moderate pressure gives a significantly smaller maximum and mean elastic modulus than when measuring with hard pressure.

Results showed that there were significant differences ($p < 0.05$, Table 2) in the maximum value of the elastic modulus of the RF and PT when different ROI's size was used. Further evaluation of the muscle using post-hoc Dunn's multiple comparison tests indicated that a significantly smaller maximum elastic modulus of RF resulted from using the 8 mm diameter ROI's size, than that of using the 10 mm and 12 mm diameter ROI's size, while a significantly smaller maximum elastic modulus of RF was observed from using the 10 mm rather than the 12 mm diameter

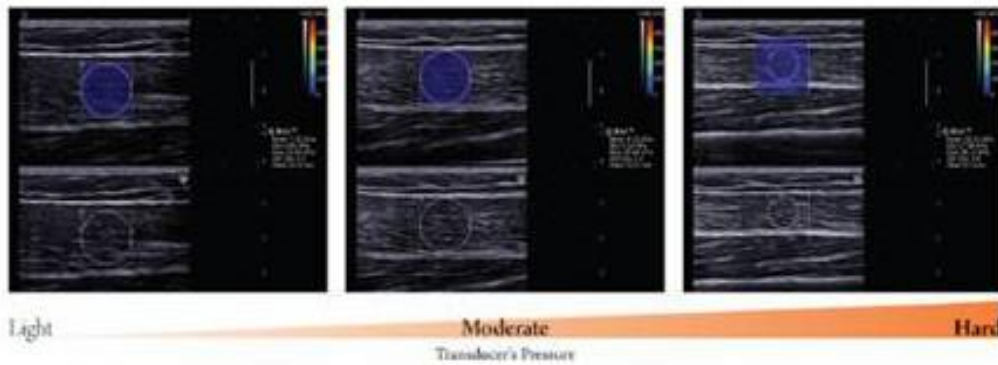


Figure 1. Longitudinal sonograms of rectus femoris (RF) taken with different transducer's pressure. Upper images show color-coded box presentations of RF elasticity (stiffer areas were coded in red and softer areas in blue) superimposed on a longitudinal grey scale sonogram of RF, with the circle representing the region of interest and its corresponding elastic modulus demonstrating under Q-Box™ on the right. Bottom images show longitudinal grey scale sonograms of RF on the identical scan planes. Transducer' pressure changed gradually from light to hard. The transducer was kept motionless for 8 to 12 seconds during the acquisition of the SWUE sonogram and ROI of 15 mm diameter was used. doi:10.1371/journal.pone.0044348.g001

ROIs size. Further evaluation of the tendon using post-hoc Dunn's multiple comparison tests indicated that a significantly smaller maximum elastic modulus of PT resulted from using the 2 mm diameter ROI's size, than that of using the 3 mm and 4 mm diameter ROI's size. However, there was no significant difference in the mean value of the elastic modulus of the RF and PT when different ROI's size was used.

There were no significant differences in both the maximum and mean value of the elastic modulus of the RF and PT with different acquisition times of the SWUE sonogram were used ($p > 0.05$).

Discussion

SWUE is a new real time ultrasound imaging mode that quantitatively measures the elastic modulus of local tissue. This

new mode appear to be a promising tool to improve understanding of the elastic properties of musculoskeletal tissues, which facilitates diagnosis and evaluation of degenerative myopathies, and assists in determining the best rehabilitation program for sports injuries patients, stroke patients and diabetes [8,14]. SWUE demonstrates not only the traditional colour-coded images of tissue hardness, superimposed on a grayscale sonogram, on all striated muscles and superficial tendons (provided that the transducer can be placed on their surface), but also quantitatively presents the colour scale with the maximum and mean elastic modulus values expressed in kPa. Previous studies have demonstrated that SWUE is capable of estimating the elastic modulus of muscle, but to the best of our knowledge, there has been no standardization of SWUE's technical settings, which has hindered its clinical applications. The results of this study indicated that

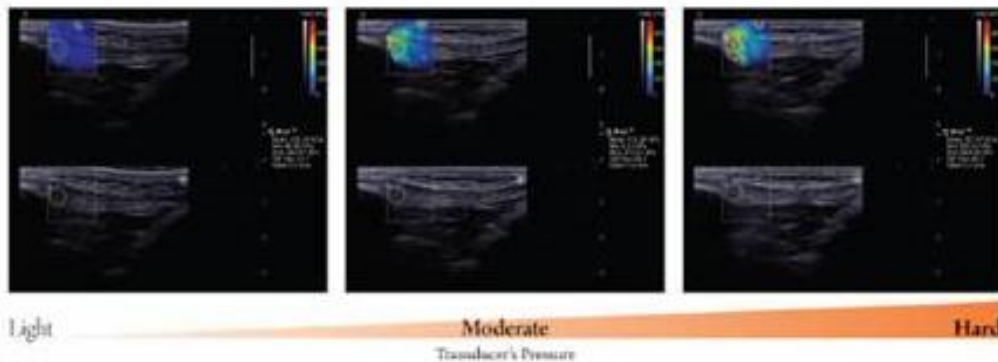


Figure 2. Longitudinal sonograms of patella tendon (PT) taken with different transducer's pressure. Upper images show color-coded box presentations of PT elasticity (stiffer areas were coded in red and softer areas in blue) superimposed on longitudinal grey scale sonograms of PT, with the circle representing the region of interest and its corresponding elastic modulus demonstrating under Q-Box™ on the right. Bottom images show longitudinal grey scale sonograms of PT on the identical scan planes. Transducer' pressure changed gradually from light to hard. The transducer was kept motionless for 8 to 12 seconds during the acquisition of the SWUE sonogram and ROI of 4 mm diameter was used. doi:10.1371/journal.pone.0044348.g002

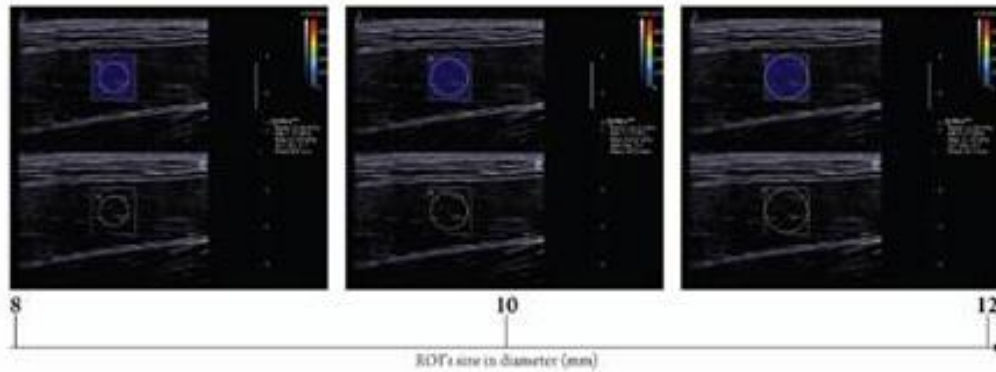


Figure 3. Longitudinal sonograms of rectus femoris (RF) with elastic modulus measured by various region of interest (ROI) sizes. Upper images show color-coded box presentations of RF elasticity (stiffer areas were coded in red and softer areas in blue) superimposed on a longitudinal grey scale sonogram of RF, with the circle representing the region of interest and its corresponding elastic modulus demonstrating under Q-Box™ on the right. Bottom images show longitudinal grey scale sonograms of RF on the identical scan planes. Transducer pressure applied was light for 8 to 12 seconds during the acquisition of the SWUE sonogram. doi:10.1371/journal.pone.0044048.g003

variations of elastic modulus of muscle and tendon were found when different transducer's pressure and region of interest (ROI)'s size were applied, which might possibly reflect the presence of measurement error due to altered/unstandardized technical settings of SWUE.

There were significant differences in both the maximum and mean value of the elastic modulus of the RF and PT when different transducer pressures were applied. In addition, the elastic modulus of the RF and PT increased with increasing transducer's pressure. With greater pressure exerted on the skin, stiffness of the RF and RT was affected since the sum total elastic modulus consists of the pressure by the external load, the underlying subcutaneous fat, as well as those of the muscle and tendon themselves. In contrast to the traditional ultrasound elastography

technique, which requires deformation/compression of targeted tissues to produce strain within the tissue resulting in different grades of elasticity displayed over the grayscale sonogram [15,16], SWUE produces an elastography sonogram based on the combination of a radiation force induced in a tissue by an ultrasonic beam and an ultrafast imaging sequence capable of capturing the real-time propagation of the resulting shear waves [11]. Therefore, no external load was required in SWUE, as the operator's compression pressure strongly and directly affects the investigated tissues properties, leading to an error in the resulting elastic modulus. We support the manufacturer's suggested technical setting that the operator should only induce light pressure with the transducer, at the surface of the skin with a generous amount of coupling gel.

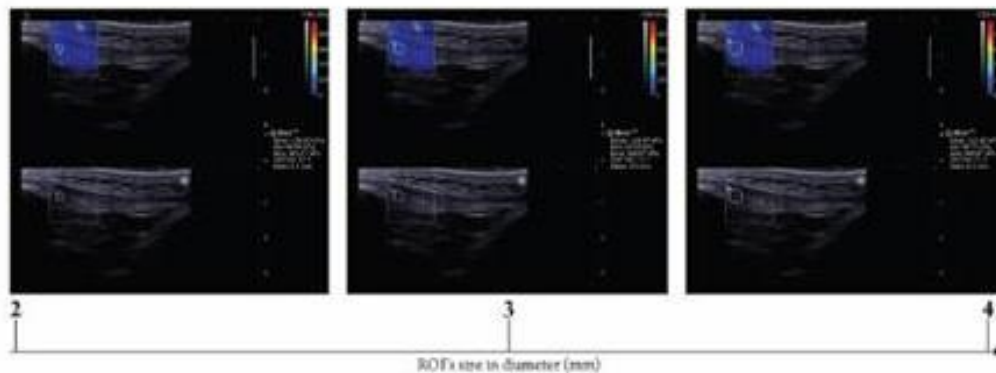


Figure 4. Longitudinal sonograms of patella tendon (PT) with elastic modulus measured by various region of interest (ROI) sizes. Upper images show color-coded box presentations of PT elasticity (stiffer areas were coded in red and softer areas in blue) superimposed on a longitudinal grey scale sonogram of PT, with the circle representing the region of interest and its corresponding elastic modulus demonstrating under Q-Box™ on the right. Bottom images show longitudinal grey scale sonograms of PT on the identical scan planes. Transducer pressure applied was light for 8 to 12 seconds during the acquisition of the SWUE sonogram. doi:10.1371/journal.pone.0044048.g004

Table 1. Comparison of the maximum and mean value of Elastic modulus of RF and PT when different transducer's pressure was applied.

	Maximum value			p-value	Mean value			p-value
	Mean ± SD (kPa)				Mean ± SD (kPa)			
	Light	Moderate	Hard		Light	Moderate	Hard	
Muscle	40.12±23.65	46.29±23.00	50.64±28.67	<0.05	12.78±3.56	18.51±6.71	32.39±14.17	<0.05
Tendon	100.23±44.61	102.51±79.66	20.927±122.21	<0.05	69.80±23.14	107.33±53.63	134.57±73.75	<0.05

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Results of the present study showed that there were significant differences in the maximum value of the elastic modulus of the RF and PT when different ROI's size was used. In addition, the maximum elastic modulus of the RF and PT increased with increasing ROI's size. The shape of the ROI in the ultrasound unit was a circular structure by default, with a range of diameters (2–12 mm) for the measurement of the elastic modulus of the targeted region/tissue, placed at the center of the SWUE acquisition box. The larger the ROI's size, the higher the chance to include the muscle fascia and dense collagen fiber, which accounted for the maximum value of the elastic modulus within the SWUE acquisition box. Previous studies with the application of SWUE on liver, breast and thyroid focused solely on the differentiation of the elastic modulus between benign and malignant lesions [17–19], therefore the size of the ROI was determined by the area of the suspected malignancy. However, in the musculoskeletal system, clinicians/physiotherapists are interested in understanding the degree of musculoskeletal spasticity and treat the entire affected musculoskeletal tissue as a whole [8]. In contrast to the maximum value of elastic modulus, no significant difference in the mean value of the elasticity modulus of the RF and PT was found, when different ROI's size was used, due to the averaging of the elastic modulus in the different sizes of ROI. Therefore, it is preferable to report the mean value of the elastic modulus of the muscle and tendon when using different ROI's size across different scanning sessions.

No significant difference was found in both the maximum and mean value of the elastic modulus of the RF and PT when comparing different acquisition times of SWUE sonograms. Previous studies using SWUE had a great discrepancy on the determination of the acquisition time of SWUE sonograms, ranging from 5 seconds [14] to 10–20 seconds [19], which differed from the manufacturer's suggested duration (8–12 seconds). Although there was no significant difference found in both the maximum and mean value of the elastic modulus of the RF and PT with increased acquisition time up to 20

seconds, the operator should be cautious of the transducer's positioning since there may be a position shift of the transducer when acquisition time increases, which would affect the measurement of the elastic modulus of the affected tissues since the imaged area may no longer be the original affected area.

The present study evaluated the variations of the elastic modulus of the muscle and tendon when different technical aspects were altered: transducer's pressure, region of interest (ROI)'s size, and acquisition time, using shear wave ultrasound imaging. However, other factors such as the underlying complex mechanical properties of musculoskeletal tissues, and how neuromuscular diseases would affect the stiffness of musculoskeletal tissues, were not evaluated in the study. Further studies to establish the norm of the elastic modulus of musculoskeletal tissues in healthy subjects, elite athletes and patients with various muscle injuries are suggested. Transducer's pressures were subjectively applied in the present study, which limited the quantitative evaluation on its effect on pre-compression. Although all the ultrasound examinations were performed by a single operator, and constructive technique by focusing on the deformation of structures to ensure constant different pressures was applied, further studies on quantifying the transducer's pressure applied and its effect on elastic modulus are suggested.

Conclusions

SWUE is a useful imaging technique in evaluating the variations of the elastic modulus of the muscle and tendon. The SWUE on the muscle and tendon should be performed with the lightest transducer's pressure, a shorter acquisition time for the SWUE sonogram, while measuring the mean Elastic modulus regardless the region of interest (ROI)'s size. Considerable action should be taken in the standardization of various technical settings before obtaining meaningful data for diagnosis and for guiding corrective therapy.

Table 2. Comparison of the maximum and mean value of Elastic modulus of RF and PT when different ROI's size was used.

	Maximum value			p-value	Mean value			p-value
	Mean ± SD (kPa)				Mean ± SD (kPa)			
	8 mm	10 mm	12 mm		8 mm	10 mm	12 mm	
Muscle	25.92±9.22	302.2±14.21	36.68±18.86	<0.05	12.78±3.53	12.77±3.40	126.8±23.55	0.92
	2 mm	3 mm	4 mm		2 mm	3 mm	4 mm	
Tendon	86.67±43.59	922.0±42.25	97.38±43.53	<0.05	72.48±36.45	69.98±33.58	700.0±232.11	0.47

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Author Contributions

Conceived and designed the experiments: BK. Performed the experiments: BK. Analyzed the data: BK, ZJZ, AI. Contributed reagents/materials/

analysis tools: BK, VI. Wrote the paper: BK. Manuscript editing: BK, SNF, VI.

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APPENDIX VI

Poster in 2011 World Federation for Ultrasound in Medicine and Biology

but US findings are not sufficiently convincing for the operator. We show US findings correlated with MR findings to provide enough conviction to the US operator.

Imaging Findings or Procedure Details: 1.The forearm is supinated and placed on the thigh for evaluation of the long head of the biceps. 2.The arm is externally rotated, to bring the tendon into a more anterior position for evaluation of subscapularis tendon. 3.The hand is placed on the buttock with the elbow pointed backward for evaluation of supraspinatus tendon. 4.The arm is bent in front of the chest and held contralateral to the shoulder for evaluating the infraspinatus and postglenoid fossa. 5.Dynamic examination is performed during lateral passive elevation of the arm for evaluation of the subdeltoid bursa wall thickening or adhesion. 6. An acromioclavicular joint view is obtained.

Conclusion: High-resolution US is a very useful modality for rotator cuff and non-rotator cuff disease. Especially on dynamic US, adhesive capsulitis can be diagnosed by restriction of the sliding movement of the supraspinatus tendon under the acromion during the abduction of the arm. Ultrasonography findings of the rotator cuff and non-rotator cuff lesions are usually according to MR findings.

Musculoskeletal/ Varia, Rheumatology

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Development of Puncture Needle-Type Acoustic Impedance Measurement System for Bone Density Measurement by Ultrasonic Interference Method

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Purpose: The purpose of this study is to develop a puncture needle-type bone density measurement system.

Material & Methods: This system consists of a fused quartz fiber as a transmission line of the ultrasound attached with a transducer. The fiber is inserted in a puncture needle. The puncture needle is inserted to the body, and then the fiber is pushed out of the needle and contacts with the surface of the bone through soft tissue. The acoustic impedance of the bone is obtained by the ultrasonic interference method.

Results: A fused quartz fiber (0.88 mm diameter, 161 mm length) was connected to a transducer. An electrical burst wave (7 cycle, 50 V_{pp} amplitude, 8.0 MHz center frequency) was applied. The fiber was in-

Wonju/KR

Purpose: Skeletal muscle atrophy typically results from reduced muscle use, as in unloading, bed rest, denervation, and space flight. Skeletal muscle cells are susceptible to oxidative stress induced through electron transport and oxygen flux during normal contraction, and this stress may increase with exercise intensity. Therefore, an understanding of vibration, ultrasound, light and chemical therapy stimulator is important in terms of developing methods for treating skeletal muscle damage. We investigated the effects of KTJ416 on muscle disorders and the underlying mechanism of oxidative stress-induced C₂C₁₂ skeletal muscle myoblast damage.

Material & Methods: To assess the protective effects of KTJ416 on oxidative stress-induced C₂C₁₂ skeletal muscle myoblasts, we measured the viability of the cells, showing that KTJ416 pre-treatment significantly reduced the decreased cell viability after H₂O₂ treatment. We also investigated the mechanism of this protective effect of KTJ416.

Results: In Western blot analysis, the heat shock protein-70 levels increased significantly in the KTJ416-pretreated myoblasts. We used high performance liquid chromatography to examine the level of endogenous ceramide after pre-treatment with KTJ416 followed by exposure to H₂O₂. While hydrogen peroxide increased the ceramide content to approximately 166% of the control level, pre-treatment with KTJ416 inhibited this increase, maintaining the ceramide content at the control level.

Conclusion: We demonstrated that KTJ416 regulates ceramide levels to protect against oxidative stress-induced C₂C₁₂ muscle myoblast damage. We suggest the potential benefits in the treatment of oxidative stress-related muscle disorders.

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ShearWave Ultrasound Elastography of Thigh Muscles:

Intra- and Inter-Rater Reliability

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Purpose: The objective of this study was to investigate the intra- and inter-rater reliability of thigh muscles using ShearWave Ultrasound Elastography (SWUE).

Material & Methods: 11 healthy subjects (8 male, 3 female; age: 26.1 ± 3.2 years; weight: 58.7 ± 12.3 kg; height: 169.2 ± 10.0 cm) were recruited. Four thigh muscles were assessed by SWUE (Aixplorer; Supersonic Imaging, Aix-en-Provence, France) by two raters within a day and one hour apart; and by the first rater twice with 3 hours apart. The thigh muscles being evaluated were rector femoris (RF), vastus lateralis (VL),

Oral presentation in

2013 Student Conference in Sports Sciences, Medicine and Rehabilitation

Abstracts

CHANGES IN ELASTIC PROPERTIES OF PATELLAR TENDON IN ATHLETES WITH UNILATERAL PATELLAR TENDINOPATHY AND ITS RELATIONSHIP WITH FUNCTIONAL ABILITIES AND PAIN.

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Background:

Patellar tendinopathy (PT) is one of the most common musculoskeletal disorders among athletes related to in jumping sports. The tendinopathy may influence the elastic properties of tendon and pain as well as functional abilities. The purposes of this study were to compare the elastic properties of healthy and patellar tendon with tendinopathy in athletes with unilateral PT and examine its relationship with the intensity of pain and functional abilities.

Methods:

Fourteen male athletes participated in jumping sports aged between 18 and 31 with unilateral PT were enrolled. The morphological and elastic properties of the patellar tendon were assessed by the grey and elastography mode of an Ultrasound machine (Supersonic Imaging, Aix-en-Provence, France). The Victorian Institute of Sport Assessment-patella (VISA-p) scale was used to evaluate functional abilities of athletes as well as the intensity of pain during certain activities.

Results:

Significantly higher shear elastic modulus (from 15.74 kPa to 85.64 kPa; by 46.8%), thicken tendon (from 0.55cm to 1.04cm; by 36.0%) and larger CSA (from 1.16cm² to 2.18cm²; by 14.7 %) were found in the patellar tendon with tendonopathy when compared the healthy side (using paired tests, all p<0.05). Significant correlations were identified between side-to-side differences in patellar tendon shear elastic modulus and the VISA score ($\rho=-0.81$; p<0.05); as well as the intensity of pain during down stairs, lunging and single leg jumping (ρ ranged from 0.69 to 0.77; p<0.05).

Discussion & Conclusion:

The patellar tendon with tendonopathy is stiffer, thicker and larger when compared with the non-painful side. Side-to-side difference in the elastic modulus is related to dysfunction and pain perceived from the athletes during some common activities.



Programme

The 3rd International Scientific Tendinopathy Symposium (ISTTS) will run from Friday 5th to Saturday 6th September 2014, followed by an optional Tendinopathy clinical workshop on Sunday 7th September. The scientific programme is available here: [ISTTS_Programme_2014.pdf](#).

Plenary Speakers:

Translational

- The aetiology of tendinopathy: George Murrell (Sydney)
- Pain therapies: Paul Ackermann (Stockholm)
- Drug discovery for tendon repair and regeneration – a novel frontier: Olivier Leupin (Basel)
- Genetics of tendinopathy: Malcolm Collins (Cape Town)

Basic Science

- Cell biology and growth factors: Britt Wildemann (Berlin)
- Tendon stem / progenitor cell and its role in the development of degenerative tendinopathy (co-sponsored ISL&T): James Wang (Pittsburgh)
- Promoting repair in fatigue damaged tendons: Nelly Andarawis-Puri (New York)
- Synthesis and assembly of the collagenous extracellular matrix in tendon: Liz Laird (Liverpool)
- Mechanotransduction - The tenocyte and its response to strain in a stressful environment: Graham Riley (Norwich)

Clinical Pathways

- Stem cell therapy for tendinopathy – what is the evidence from animal



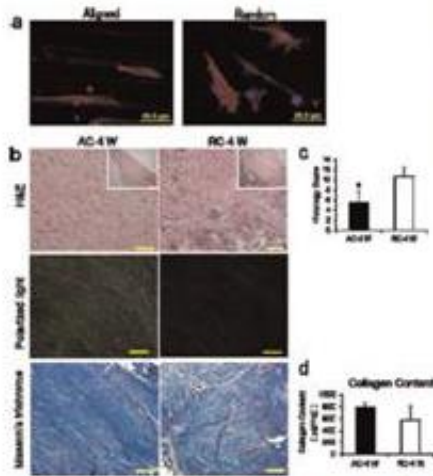
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Abstracts

SDF-1. In rat Achilles tendon repair model, AC-treated tendon had superior structural and mechanical properties than RC-treated tendon. Cell labelling and extracellular matrix expression assays demonstrated that the transplanted hiPSC-MSCs contributed directly to tendon regeneration. Moreover, no teratoma was found in any samples. These findings present a strategy combining well-aligned fibre scaffold with iPSC-MSCs for tendon regeneration and may assist in clinical regenerative medicine to treat tendon diseases.



Abstract 112 Figure 1 (a) Confocal micrograph of F-actin exhibiting elongated hiPSC-MSCs on the aligned scaffold and morphological change of hiPSC-MSCs on the randomly-oriented scaffold. (b) Histology morphology of the repair tendon after 4 weeks post-surgery. (c) Histology score of repaired tendon. (d) Quantitative analyses of the collagen content in the repaired tendon.

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113 CHANGES IN STIFFNESS OF PATELLAR TENDON IN ATHLETES WITH TENDINOPATHY AND THEIR RELATIONSHIPS WITH PAIN AND DYSFUNCTION

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Introduction Patellar tendinopathy (PT) is one of the most common knee disorders among athletes. Changes in elasticity of the painful tendon and how these relate to the pressure pain and dysfunction remain unclear. Therefore, the present study aimed to compare the elastic properties of patellar tendons between athlete with and without unilateral PT and to examine its association with pressure pain and dysfunction.

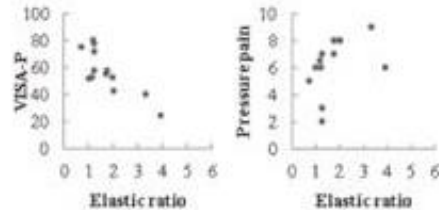
Method Thirteen male athletes with unilateral PT (age 22.9 ± 4.6 years, height 1.8 ± 0.5m and weight 76.2 ± 6.3 kg, duration

of symptom 22.6 ± 22.3 months) and 20 healthy control subjects (age 24.9 ± 4.4 years, height 1.82 ± 0.60m and weight 73.4 ± 7.9 kg) joined in this study. Supersonic shear imaging technique was used to measure the elastic properties of the proximal part of the patellar tendon (SuperSonic Imagine, Aix-en-Provence, France). The mean value of Young's modulus within the region of interest (Figure 1) was computed by the ultrasound system. Shear elastic modulus was calculated by dividing the Young's modulus by 3 (Royer et al. 2011). The ratio of mean shear elastic modulus between the painful and non-painful knee was expressed as elastic ratio in athletes with unilateral PT. The intensity of pressure pain and dysfunction were quantified with a 10-lb force to the most painful site and the Victorian Institute of Sport Assessment-patella (VISA-P) questionnaire (Visentini et al. 1998), respectively.

Results In athletes with unilateral PT, the painful tendons had higher shear elastic modulus (43.6 ± 17.9 kPa) than the non-painful side (25.8 ± 10.6 kPa) (by 68.9%; $p < 0.05$) or the dominant side of the healthy controls (27.5 ± 11.3 kPa) (by 58.5%, $p < 0.05$). Significant correlations were found between tendon shear elastic modulus ratio and the intensity of pressure pain ($r_{ho} = 0.62$; $p = 0.024$) and VISA-P scores ($r_{ho} = -0.61$; $p = 0.026$) (Figure 2).



Abstract 113 Figure 1 A representative image obtained from the supersonic shear imaging technique on a patellar tendon with tendinopathy



Abstract 113 Figure 2 Correlation between tendon elastic ratio and VISA-P score and pressure pain

Discussion Athletes with unilateral PT had stiffer patellar tendon on the painful side than the non-painful side and the dominant side of healthy athletes. A higher ratio of elastic modulus between the painful and non-painful knee is related with a greater intensity of pressure pain and a lower VISA-P score. Such findings suggest that an increase in tendon stiffness in athletes with unilateral patellar tendinopathy is associated with the tendon pain and dysfunction.

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