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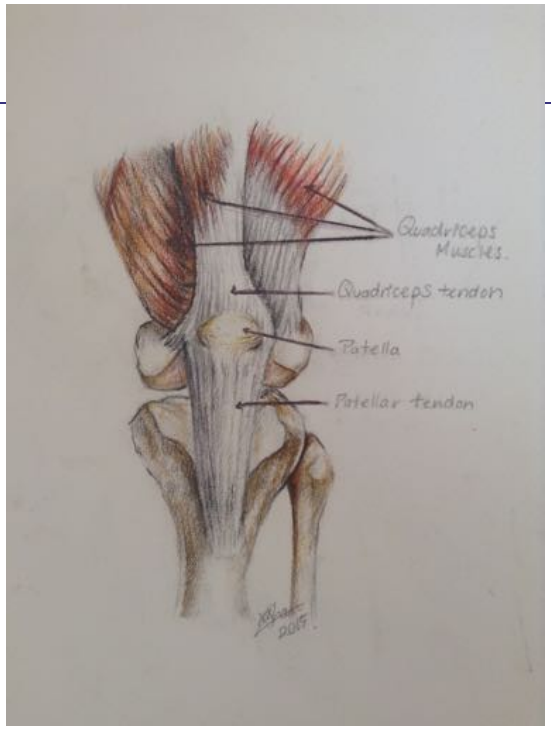
FACULTY OF HEALTH SCIENCES



Ph.D. Thesis

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Patellar Tendon Development in Adolescent Dancers

Quantifying normal and abnormal tendon development

Supervisors: Michael Kjaer, Peter Magnusson, and Jill Cook

This thesis has been submitted to the Graduate School of Health and Medical Sciences, University of Copenhagen

Submitted on: 31 January, 2018

Name of department:	Institute of Clinical Medicine, Basic and Clinical Research in Musculoskeletal and Oral Sciences
Author(s):	Aliza Chana Rudavsky, DPT
Title and subtitle:	Patellar Tendon Development in Adolescent Dancers; Quantifying normal and pathological tendon development
Topic description:	Utilizing novel imaging technology, Ultrasound Tissue Characterization, this thesis study described and quantified the changes that occur in normal proximal patellar tendon during the adolescent growth spurt. It also described the development of pathology within the proximal patellar tendon during this stage.
Supervisor:	Michael Kjaer, Peter Magnusson, and Jill Cook
Submitted on:	31 January, 2018

Preface and Acknowledgements

This thesis is based on experimental work performed in a joint collaboration between the Department of Physical & Occupational Therapy and Institute of Sports Medicine, Copenhagen University Hospital, Bispebjerg and Faculty of Health and Medical Sciences (University of Copenhagen, Denmark) and the Department of Physiotherapy (Monash University, Melbourne, Australia) between 2015 and 2018.

I would like to thank Dr. Jill Cook for inspiring this project, investing many years of time and energy to help me see it through and supporting me from the opposite side of the world. You are a constant source of inspiration to me, you've taught me everything I know about tendons and I am eternally grateful that you took a chance on me. A huge thanks to Dr. Sean Docking who taught me everything about UTC and spent countless hours going through scans and drafts with me. You are an inspiring researcher with brilliant ideas and abundant knowledge. Thank you for supporting and encouraging me throughout these many years. A special thanks to Dr. Michael Kjaer and Dr. Peter Magnusson who have supported this research and taken a chance on this unique collaboration. It has been an honor to get to know you and your work. Your feedback and guidance throughout this process have been critical.

I am eternally grateful to Janet Karin, Margaret Wilson and all of the students who participated in this study. Without them, this would not have been possible. It was also a pleasure to see these students grow up and change during their adolescent years. You are all my inspiration.

To my family, especially my husband, Danny: there is no way I could have done this without you. Thank you for the countless meals you cooked while I stared at the computer, for repeatedly helping me with my statistical questions, and for encouraging me when I needed a pep-talk. My mother would have been the proudest person to see this project finished so I dedicate this thesis to her.

Lastly, I would like to acknowledge the original cover artwork, which was illustrated by Shan Shan Zhang, Charles Sturt University, Australia

Manuscripts

1. **Title:** Characterizing the proximal patellar tendon attachment and its relationship to skeletal maturity in adolescent ballet dancers.
   Summary: This cross-sectional study examined and compared the tendon appearance of the proximal patellar tendon attachment of pre, peri, post pubertal elite ballet dancers and adults. Outcomes included ultrasound appearance and classification on grey scale imaging, internal tendon structure using ultrasound tissue characterization (section 1.4) and tendon thickness. Measures were compared with skeletal maturity, which was estimated by peak height velocity (section 3.2e). Findings showed that mid-portion tendon thickness increased with skeletal maturity and variance in structural appearance was greater in pre and peri-peak height velocity participants. One-year after peak height velocity, tendon attachment appeared similar to adult tendons indicating that adolescence may be a critical time for the formation of normal tendon attachment.

2. **Title:** Quantifying proximal patellar tendon changes during adolescence in elite ballet dancers, a 2-year study.
   Summary: This prospective study monitored proximal patellar chances in 52 elite adolescent dancers every 6 months for 2 years. Changes in tendon size (anterior-posterior diameter) and echogenicity, measured using ultrasound tissue characterization, were collected at each time point. Peak height velocity was measured each time to estimate skeletal maturity. Findings showed that following peak height velocity, the proximal patellar tendon attachment increased in thickness and demonstrated a more stable echopattern representative of aligned fibrillar structure

3. **Title**: Proximal patellar tendon pathology can develop during adolescence in young ballet dancers- a 2 year longitudinal study.
   Summary: The aim of this prospective study was to follow adolescent ballet dancers to identify if pathology develops and determine its relation to the adolescent growth spurt. This study included 57 adolescent ballet students who were monitored for 2 years. Normal and pathological tendon changes were measured with an ultrasound scan and ultrasound tissue characterization to quantify intra-tendinous changes. Participants also reported any injuries or dance modifications as well as tendon pain with the VISA-P and single leg decline squat (section 3.2f). The findings of this study showed that 9% of adolescent dancers developed pathology during this study and that these changes were not associated with pain.

Table of Contents

1. Introduction

1.1 General Intro:

Patellar tendinopathy, commonly known as jumper's knee, is a clinical diagnosis of pain and dysfunction in the proximal patellar tendon. It typically affects jumping athletes from adolescence through to the fourth decade of life. While this is not a condition that often requires surgery, it still has a significant impact and burden on health and quality of life by limiting sports and activity participation for recreational athletes. It can even be career ending for professional athletes [1].

A key risk factor for developing patellar tendinopathy is the presence of pathology at the proximal patellar tendon, which is identified on ultrasound or magnetic resonance imaging [2-5]. Throughout this dissertation, the term tendinopathy will refer to pain associated with the tendon, while the term tendon pathology will be used to indicate histopathology or abnormal imaging without reference to pain.

Little is known about how or when pathology develops in the tendon, though it can be seen in adolescent tendons as young as 14-16 years old [3-5]. Core tendon tissue becomes relatively inert around 17 years of age as demonstrated by a Carbon-14 bomb-pulse method study [6]. This study showed that the core of tendon tissue in the Achilles is formed during adolescence and does not show very much turnover after approximately 17 years of age.

There is still turnover in tendon collagen later in life, perhaps associated with excess load and pathology [7]. When pathology develops, especially in the tendon core, is unknown and may vary between tendons, however it may develop prior to or during adolescence and may remain in the tendon through adulthood. Once pathology develops on the tendon, the athlete is at a higher risk of developing pain in adulthood, as the pathologic area is likely to remain in the tendon.

To date, no prospective studies have investigated how the proximal patellar tendon changes through early to late adolescence though this may be a critical stage for both normal and pathological tendon development. As the presence of pathology in the patellar tendon increases an athlete's risk of developing pain four-fold [4], it is important to clarify when the pathology initially develops. Due to our limited understanding, it is difficult to guide sports coaches or even rehabilitation specialists on appropriate injury prevention strategies. By gaining an understanding of both normal and abnormal tendon development, future research can extend into prevention strategies to minimize the onset of pain and dysfunction in recreational and professional jumping athletes.

1.2 Incidence of pathology and tendinopathy in adolescents, dancers, and impact on athletes of other sports:

There is limited research looking at patellar tendon pain and pathology in young athletes. A systematic review of lower limb (patellar and Achilles) tendinopathy in adolescent athletes participating in a variety of sports, reported tendinopathy in 8-33% of athletes ranging from 14-20 years old [8]. In young basketball players between 14-18 years old, 26% had hypoechoic areas within their patellar tendons on ultrasound and 7% had patellar tendinopathy symptoms [9].

No studies have looked at patellar tendon pathology development in young adolescent athletes, however, one study shows that only two of 25 normal tendons (8%) developed

tendinopathy symptoms after the age of 16 in a school for elite volleyball players [10]. In this cohort of 44 tendons, where participants were age 15-16 at the start of the study, 36 tendons were asymptomatic, however, 20% of those asymptomatic tendons already had pathology at baseline. Eight tendons were symptomatic at the start with 88% containing pathology that developed before 15-16 years of age [10]. This finding supports the hypothesis that pathology can develop in young athletes and may be a risk factor for the development of symptoms later on.

Patellar tendon abnormalities on ultrasound are three times more common than clinical symptoms of tendinopathy [9] and may be a precursor in developing symptoms. To date, one study has followed symptom development in dancers longitudinally and found that 12% of elite ballet dancers showed moderate to severe hypoechoic areas on ultrasound at baseline [2]. Of that 12% who showed pathology, only 16% of these dancers went on develop symptoms over a 2-year study[2]. These findings suggest that the presence of a hypoechoic area on ultrasound is a risk factor for going on to develop symptoms, however in this study, many tendons did not develop symptoms during the investigation period. As research on ballet dancers is limited, it is important to also look at the impact of pathology on athletes of other jumping sports.

In elite basketball players, 32% of asymptomatic players showed pathology on imaging [11]. However, 83% of elite volleyball players who presented with patellar tendinopathy also had pathology within their tendons [5]. Pathology was present for 36.1% of elite rugby players on their patellar tendons on ultrasound [12], while in elite ballet dancers, 12% showed moderate to severe hypoechoic areas on ultrasound that was a risk factor for developing pain over a 2-year period [2]. While not all athletes with pathology will develop pain, most with pain will also have pathology.

The prevalence of patellar tendinopathy symptoms is highest in jumping and cutting sports as these are the activities that maximally load the patellar tendon. In the non-elite sports population, rates of patellar tendinopathy across a variety of sports include: 14.4% of volleyball players, 11.8% of basketball players, 13.3% of handball players and 2.5% of soccer players [13]. In elite junior basketball players, 36% had patellar tendinopathy [14] and 13.3% of elite rugby players were diagnosed with patellar tendinopathy [12].

1.3 Patellar tendon development:

Current understanding of how the proximal patellar tendon matures is based on limited literature describing general tendon development. Post-natal tendons are known to elongate and thicken at a rate proportional to the growth of their associated muscles and bones [15-17]. There may, however, be a delay or imbalance between growth rates of tendons compared to their associated muscles during puberty due to musculoskeletal changes during this stage [18].

Puberty, and specifically the adolescent growth spurt, is a period of rapid growth, especially of the long bones in the skeleton. Because of this quick elongation of long bones and subsequent tensile strain on tendons, puberty may be a critical time for tendon attachment formation. Puberty can be quantified by measuring the peak growth of the adolescent growth spurt (peak height velocity (PHV)). Peak height velocity is typically achieved around 12 years old for girls and 14 for boys [19]. Puberty may be a crucial time to assess the proximal patellar tendon attachment as tendon tissue matures towards the end of puberty due to findings that tendon collagen has limited turnover after age 17 [6].

Tendon changes during development have thus far been measured by quantifying tendon thickness or cross sectional area (CSA) using conventional greyscale ultrasound. Mersmann et.al, reported muscle-tendon imbalance seen in adolescent athletes approximately 16 years old, who demonstrated quadriceps strength and morphology consistent with adult athletes

but demonstrated smaller CSA of the patellar tendon as compared to adults [18]. A cross sectional study demonstrated patellar tendon thickness was positively correlated with age in 11-16 year old elite volleyball players; r=0.688 for proximal tendon thickness and r=0.281 for distal tendon thickness [20]. However, no significant difference in patellar tendon thickness was found in participants younger than, versus older than, 13-year-old athletes from a variety of sports including: ball, combat and water sports, combined disciplines, cycling, and controls [21]. Due to these varied results from tendon maturation studies, there is still some uncertainty as to when and how normal patellar tendon maturation occurs. This also suggests that the load on the tendon may not influence development, as low patellar tendon load sports such as cycling were not different from higher load sports such as combat sports.

To date, studies looking at patellar tendon development have been limited to greyscale ultrasound. In order to describe the distal patellar tendon appearance during puberty, Ducher et al developed an image-based staging criteria of the attachment on greyscale ultrasound to investigate its relationship to puberty, using the Tanner scale and peak height velocity to measure puberty (discussed further in section 3.2e) [22, 23]. In these studies, the authors described normal (asymptomatic) tendon development in stages: Stage 1 (immature attachment) consists of apophyseal cartilage, with or without interspersed ossicles. Stage 2 shows progressive collagen attachment onto the bone while some cartilage is still present as well. Stage 3 is a mature attachment and there is no visible cartilage at the attachment. They also showed that disruption of the normal tendon maturation process may lead to the development of pathology at the distal attachment [22, 23] which has been associated with pain (Osgood-Schlatter disease) [24]. These studies also demonstrated that the distal attachment of the patellar tendon transitions to a mature attachment on average 2 years after PHV[22, 23]. Because of the increased risk for the development of pain and injury in those with a pathological tendon, a better understanding of normal and abnormal tendon development is important. To date, no studies have developed a similar image-based staging for the proximal patellar tendon.

1.4 Measuring Tendons: Traditional Ultrasound Imaging and Ultrasound Tissue Characterization

The previous section described what is currently known about patellar tendon maturation and changes during puberty, however one of the main constraints in broadening the understanding of these changes is the limitation of conventional imaging modalities. Most of the literature uses diagnostic ultrasound to measure tendon thickness and cross sectional area, though some studies use magnetic resonance imaging (MRI) [25]. While MRI may be considered by some as the gold standard in measuring tendon dimensions due to its 3-dimentional capacity, it can be costly, time consuming and impractical for research studies. Ultrasound is portable, quick and more affordable than MRI and can be utilized in a clinical and sport setting. There are some limitations in using ultrasound to image and measure tendons, including inconsistent inter- and intra-rater reliability in measuring tendon dimensions. A systematic review of ultrasound studies for tendons showed “fair to excellent correlation” for combined inter and intra rater reliability, ICC 0.45-0.99, for tendon thickness, and ICC 0.58-0.92 for tendon CSA [25]. While ultrasound may be more convenient for researchers and clinicians to image tendons compared to MRI, there are limitations to ultrasound, including: reduced reliability due to operator experience, variability of transducer positioning and imaging protocols, it is also limited to two-dimensional measures [25, 26]. Additionally, tendon cross sectional area measurements have been shown to have poor reliability and accuracy on ultrasound [27]. While tendon dimensions can be quantified using ultrasound, the interpretation of the internal structural integrity is limited to subjective interpretation.

In order to improve on the more variable aspects of ultrasound, a novel imaging technology has been used increasingly in tendon research: Ultrasound Tissue Characterization (UTC). UTC uses a high-resolution ultrasound probe (SmartProbe 10L5, Terascon 2000; Teratech) that is fixed in a tracking device with a stand-off pad [28, 29]. It moves continuously and automatically along the stand-off pad which is placed on the tendon's axis. The probe takes a transverse-plane image every 0.2mm and dedicated UTC software renders a tomographic 3D image of the tendon based on the contiguous transverse images [28, 29]. The UTC algorithm analyzes the stability of the echopattern between the contiguous images and quantifies the architecture of the internal tendon matrix. It then assigns an echo-type (I-IV) to the tendon matrix depending of the stability of the echopattern across the transverse plane images. Type I represents the most aligned fibrilar structure and type IV represents highly disorganized structure [28, 29]. The design of the machine eliminates some of the issues of operator error (ie changes in transducer tilt angle causing artifact) because the transducer is fixed by the tracker and moves automatically, however there can be operator error in placing the stand-off pad correctly along the tendon. UTC does have standardized parameters, which allows for greater consistency between raters and scans.

Another advantage of UTC is that reliability has been demonstrated. Inter- and intra- observer reliability has been shown to be excellent (ICC > 0.90) for both data collection and analysis [29-31]. While UTC's sensitivity to measure change over time is being studied, in vitro studies validating sensitivity are not available as these studies are very difficult to perform. However, due its reliability and repeatability, it is a good tool for monitoring longitudinal tendon changes.

Additionally, because of the algorithm, it allows for an objective quantification of tendon structure as opposed to a subject interpretation upon which standard ultrasound relies. UTC echo-types were validated against histologic specimens of horse tendons [28]. While the sample size was small in this study (two normal and two pathological superficial digital flexor tendons), the echopattern of the tendon tissue on ultrasound was compared to in vitro samples. The results demonstrated that echo-type I is consistent with intact and aligned tendon bundles. Echo-type II showing waving fasculi with some discontinuity and less integer. Echo-type III is composed of mainly fibrillar matrix that disrupts echoes, while echo-type IV is mainly cellular matrix and fluid [28, 29] (Figures 1 and 2).

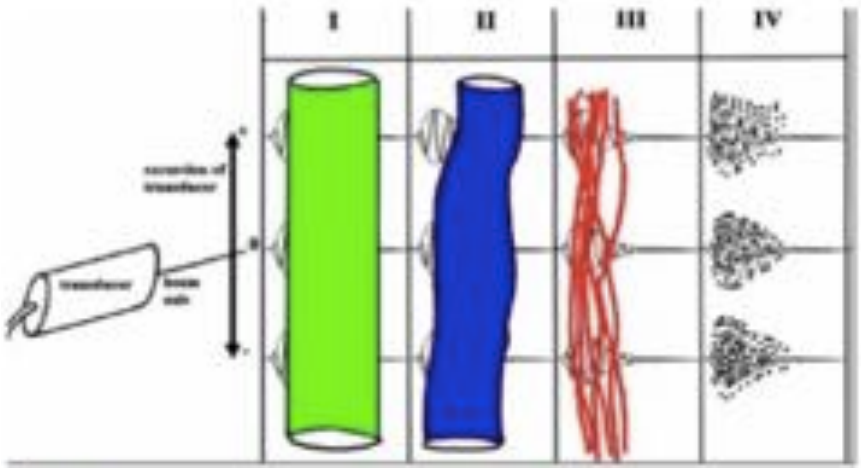
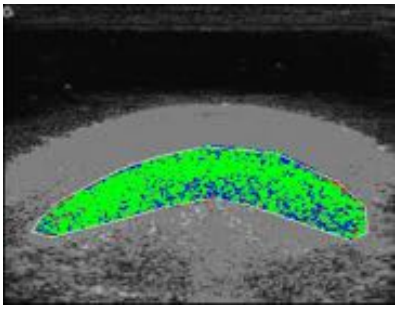
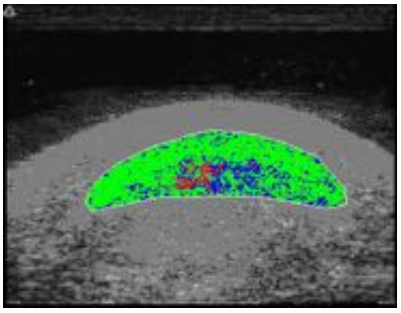


Figure 1: Ultrasound Tissue Characterization Echo-type Schema:
After the UTC algorithm analyzes the echo-patterns, it assigns a color to represent each echo-type: type I (green) intact and aligned bundles, type II (blue) increased waviness and separation

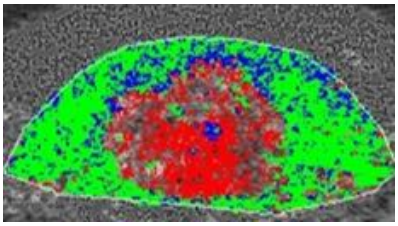
of fibrils, type III (red) decreased fibrillar integrity, and type IV (black) absence of fibrillar organization.
Adapted by permission from BMJ Publishing Group Limited. [*Ultrasonographic tissue characterisation of human Achilles tendons: quantification of tendon structure through a novel non-invasive approach.* van Schie, H.T., et al., **44**(16): p. 1153-9, 2010]
Figure 2: Ultrasound Tissue Characterization images:



2a. Normal patellar tendon with mostly type I, aligned structure.



2b. Patellar tendon with slight disorganization, type II and III



2c. Patellar tendon with highly disorganized structure.

Baseline studies have established normal UTC structural composition in adult Achilles tendons [32-34] and adolescent normal Achilles tendons [31]. In the healthy adolescent Achilles tendon there was 54.6% echo-type I, 42.8% echo-type II, 2.2% echo-type III and 0.3% echotype IV in the midportion of the tendon [31]. To date, no studies have established baseline normative values for adolescent patellar tendons on UTC.

Several studies have examined changes in tendon structure on UTC in response to short-term loading. In the Achilles tendons of Australian football players, there was a decrease in echo-type I and an increase in echo-type II 2 days after loading, and the echo-pattern returned to baseline after 4 days [34]. Patellar tendons of adolescent volleyball players participating in a tournament showed no change in UTC echo-type I and II during 5 days of cumulative loading [30]. This finding was consistent with Achilles tendons in runners that also showed no change in echo-type I and II at 2 and 4 days after running a 10k race [35].

Investigating long-term effects of loading on UTC echo-type, asymptomatic Achilles tendon's of 15 Australian football players significantly changed all four echo-types consistent with improved tendon structure over a 5 month season, while those who developed symptoms showed UTC changes that reflected increased disorganized structure [32]. In Achilles

tendons of cross country runners, a transition towards from type II to type I echopattern was seen over a 4 month running season showing tendon adaptation to loading during the competitive season [36]. With researchers utilizing UTC as a modality to monitor subtle changes in tendons, understanding of normal and abnormal tendon adaptation may become clearer.

1.5 Normal Tendon Behavior: Adaptations to Load

The patellar tendon transmits forces from the quadriceps and acts as a spring by storing and releasing elastic energy via the stretch-shorten cycle [37, 38]. It is primarily loaded with jumping and cutting tasks. General tendon adaptations to chronic loading include increased stiffness and hypertrophy [37, 39]. Habitual loading of the patellar tendon has been associated with greater cross sectional area of the proximal and distal patellar tendon in the dominant leg for fencing and badminton athletes [39], which is likely a mechanism to adapt to increased loading. No lasting changes were seen in patellar tendon structure in response to acute loading using the ultrasound tissue characterization (Section 1.4) in asymptomatic adolescent volleyball players and adult AFL players [30, 34]. Chronic loading may even stimulate improved tendon structure in healthy athletes [32, 36].

1.6 Abnormal Tendon Adaptation: Tendon pathology

Tendon pathogenesis is unknown and there are several models that attempt to describe the process [38, 40, 41]. These models include: micro and macro-trauma to the cell matrix [42], the "weak-link" theory which describes pathology arising from heterogenous distribution of loading throughout the tendon [7],inflammatory or degenerative processes[43, 44], and the tendon cell/tenocyte response model which is described by the continuum of tendinopathy[38, 45]. Histopathologic samples of tendon tissue do not demonstrate the presence of inflammatory cells [46] and histopathology of tendon ruptures presents differently than tendinopathy, which diminishes support for the micro-tear/trauma theory of tendinopathy [7, 47-49]. Pathogenesis may be caused by alterations in the signaling pathways that stimulate the extracellular matrix components in response to mechanical overload [50]. Magnusson et al, suggested that loading a tendon stimulates collagen turnover to maintain and repair tendon crosslinks, where overload can contribute to degradation with the tendon and inactivity fails to sufficiently stimulate optimal turnover or maintenance, leading to pathology [7]. The model that will be discussed primarily in this thesis is the tenocyte/continuum model of tendinopathy as it has the most overt clinical application [38], and it aligns with the collagen turnover model that supports an optimal amount of loading to stimulate collagen turnover to maintain tendon crosslinks.

The tenocyte model describes the primary role of the tendon cell as maintaining the extracellular matrix in response to its environment [38]. In response to excessive or aberrant mechanical stimuli, the tenocyte initiates a cascade of activity including cell activation and proliferation, change in collagen type and proteoglycan expression [45]. With short-term mechanical loading, the increased production of proteoglycans, including aggrecan and versican, bind water within the tendon matrix and cause an increase in thickness of the tendon, which is likely an early attempt to reduce stress by increasing CSA [51]. Once the increased abusive mechanical stimulus is removed, tendons can return to a structurally normal appearance [38]. If stress/loading is not removed from the tendon, there is an increased production of proteins and further breakdown of the matrix; there can also be neural and vascular ingrowth [38]. Eventually, there will be cell turnover and more significant disorganization of the matrix with limited capacity to reverse [38].

The continuum model describes tendon pathology in three somewhat interchangeable stages: reactive tendinopathy, tendon dysrepair and degenerative tendinopathy [38]. However, tendons can also have a combination of pathological states: ie: reactive on degenerative pathology.

It has recently been proposed that there is another stage prior to reactive tendinopathy that reflects the transient nature of tendon changes described above in response to short term loading [45]. A tendon's capacity to move forward and back along the continuum model was demonstrated in patellar tendons of basketball players [52]. Players had ultrasound scans performed each month during a volleyball season and those with reactive tendon changes progressed and regressed between degenerative tendon pathology and normal tendon appearance during the season [52]. Reactive tendinopathy is associated with the short-term response, described above, where there is a thickening of the tendon due to production of water-binding proteoglycans. Both reactive and the proposed stage prior to reactive tendinopathy reflect the transient nature of tendon changes described previously in response to short term stress [45]. At this stage, UTC echo-types cannot be directly linked to pathological states though clinically they may help to clarify the degree of tissue changes in order to guide intervention.

While it is known that pathology on imaging does not always correlate with painful patellar tendinopathy, certain factors (ie, the presence of large hypoechoic area on ultrasound) have been shown to increase the risk of developing painful patellar tendinopathy [2, 4, 5, 9]. It is important to note that in small sample sizes, there have been examples of painful tendon symptoms with apparently normal tendons, although in 50% of these tendons, they transitioned to having abnormality on imaging [53].

Tendons with pathological areas in them may also have a sufficient amount of normal tissue in the tendon to cope with loading [54]. This seems to be an adaptive mechanism to manage load even if the hypoechoic area remains in the tendon, it may be loaded without causing symptoms. This indicates that pathology can exist without symptoms and vice versa. It is unknown when pathology develops, though evidence of an early age of onset of patellar tendon pathology is bolstered by data that shows only two players developing pathology after the age of 16 in a school for elite volleyball players [10].

1.7 Onset of pain in sports:

Overload is reported as a key factor associated with pain onset for tendons [55]. Overload is defined as activity that is beyond the capacity of the tendon at that point in time. For the patellar tendon, it occurs by a sudden or substantial increase in the volume of high tendon load (jumping, deceleration, change of direction) or a return to normal volume of high tendon load following a period of relative unloading: ie, return from injury or holiday [56]. The use of energy storage and release loads involved in jumping and change of direction is characteristic of the type of overload that causes patellar tendinopathy symptoms. Other sports that do not involve energy-storage loading of the patellar tendon, ie; non-jumping sports including: cycling, running or swimming, rarely provoke patellar tendon pain; knee pain seen in these sports is typically the result of other pathologies.

While in the elite ballet population, the presence of pathology on the patellar tendon was only weakly predictive of tendinopathy [2], other studies have demonstrated a stronger predictive relationship between pathology and pain in sports with high levels of jumping [4, 5, 9]. While ballet is considered to have a high level of jumping with approximately 200 jumps per class [57], only one prospective study has looked at patellar tendinopathy in dancers [2]. If there is pathology within the tendon, the capacity of what may be reduced and it will be more susceptible to overload if not sufficiently strengthened. It has been shown, however, that even tendons with pathology on imaging can have sufficient normal tissue [58], which may reduce their risk for overload and onset of symptoms.

1.8 Summary

Pathology within the proximal patellar tendon is prevalent in jumping athletes. Once pathology has developed, it may remain within the tendon. The risk of developing painful symptoms associated with patellar tendinopathy is increased by four-fold if pathology is present, however, to date it is unclear how pathology develops. Pathology has been reported on patellar tendons of mid-adolescent athletes but studies have not investigated patellar tendon changes starting in young adolescence to identify how normal and pathological proximal patellar tendons develop.

2. Aim of the studies

The primary aim of this thesis project was to examine how the patellar tendon changed and developed through early to late adolescence and identify if pathology also developed in this period.

2.1 Study I:

The aim of this study was to compare the varied proximal patellar tendon appearances of a cross-sectional population of adolescents and adults. The adolescents were ballet students who were pre, peri and post pubertal, and the adults were skeletally mature. A secondary aim was to develop an image-based scale to describe the varied appearances and determine if tendon appearance was associated with pubertal status. The hypothesis was that there would be a relationship between tendon composition (internal structure and greyscale appearance) and pubertal status.

2.2 Study II:

The aim of this longitudinal study was to quantify proximal patellar tendon changes overtime in relationship to puberty, as measured by peak height velocity. This study also aimed to establish normal values for adolescent patellar tendon structure. The hypothesis was that the proximal patellar tendon would transition to a mature appearance throughout adolescence.

2.3 Study III:

The aim of this study was to describe the development of abnormal proximal patellar tendon in adolescence and examine factors associated with the development of tendon pathology. This study also aimed to compare intrinsic and extrinsic factors in those who did and those who did not develop pathology. The hypothesis was that pathology would be seen to develop during adolescence.

3. Description of Research Project

3.1 Methodological designs and participants:

The following section presents the study design for the overall project as well as detail the key differences between the three studies. The detailed methodological information can be found in each individual manuscript located in the end of this thesis.

3.1a Study I: Cross-sectional study

This study included 68 participants: 60 were adolescent elite ballet students and 8 were healthy adults. Within the adolescent group 35 were women and 25 were men. We used peak height velocity (PHV) to estimate and categorize pubertal status. In the adolescent group the distribution was: 23 participants were pre-PHV (greater than a year prior to PHV), 28 were peri-PHV (between 1 year before or after PHV), and 9 were post-PHV (greater than 1 year after PHV). The eight adults were all skeletally mature. UTC scans of both patellar tendons were performed on all participants. The left patellar tendon was selected for analysis for the adolescent group because no scans showed pathology on the left at baseline. A normal patellar tendon (either right or left) was selected for the adults.

Tendons were categorized as normal or abnormal based on UTC images obtained at baseline. If no focal hypoechoic area was found on any scans they were categorized as a normal tendon. A greyscale score was developed to describe and classify normal tendon appearances by tendon maturity (Section 3.2c), and all scans were analyzed and scored based on image appearance in the sagittal, coronal and transverse planes. We measured anterior-posterior thickness at the proximal patellar attachment, one and two centimeters distal.

Maturity offset and peak height velocity were calculated from anthropometric measurements for the adolescent group [19]. We analyzed the relationship between tendon appearances on the greyscale score, UTC echopattern and thickness with pubertal status (pre, peri, post-PHV or mature).

3.1b Study II: Longitudinal normal development study

We included 52 adolescent elite ballet dancers in this study (from the adolescent group in study I), ages 11-18 at the start of the study, 32 were women, 20 were men. We followed these participants for 2 years and collected data every 6 months; five time points in total. At the start of the study, 36 participants were pre-PHV and 16 were post-PHV. No participants had tendon pathology, classified by the presence of a hypoechoic area on grey-scale ultrasound, at the start of the study or at greater than one time-point throughout the study. At each time point maturity offset and peak height velocity was determined, and UTC scans of both patellar tendons were performed with analysis completed on the left patellar tendons for consistency. For each time-point, the UTC echopattern, greyscale score, and tendon thickness were quantified with changes in these parameters tested in relation to maturity offset. We also tested to see if maturity offset had a greater effect on tendon structure after dancers passed peak adolescent growth spurt.

3.1c Study III: Longitudinal pathological development study

For this study we included 57 elite adolescent ballet dancers (same cohort as adolescents in study I). We followed these participants for 2 years, collecting data every 6 months with 5 time points total. Data collection involved a UTC scan of both of their patellar tendons with analysis performed on the left patellar tendons for consistency, measuring and

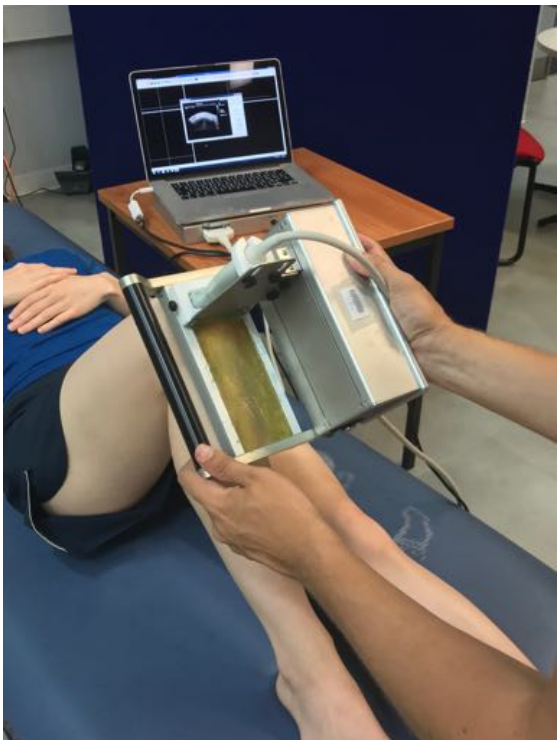
calculating maturity offset and peak height velocity, VISA-P questionnaire to assess pain and dysfunction associated with patellar tendinopathy, a single leg decline squat test for pain provocation on the patellar tendon, and detailed activity monitoring for the previous week and 6 months. All participants started with normal tendons, however throughout the course of the study five participants developed pathology within their proximal patellar tendons. Our analysis included detailed descriptions of the five pathological-tendon participants and also compared all of their outcomes with participants whose tendons remained normal.

3.2 Outcomes measures:

This section will describe each of the outcomes measures used in the studies including rationale for choosing the outcome and design for each outcome.

3.2a Ultrasound tissue characterization: Training

All three studies utilized UTC scans on the participants' left patellar tendons (Images 1 and 2). We selected the UTC instead of traditional ultrasound to be able to quantify tendon structure and compare the subtle with-in and between participant changes overtime, and to standardize the procedure for each participant over the 2 years. Aliza Rudavsky (AR), the author of this thesis and doctoral candidate, performed all of the scans and contouring throughout this study, with the exception of the scans for the final data collection point. A family emergency precluded her completing this data collection. AR was trained to use the device by UTC expert, Sean Docking (SD), PhD, who performed the scans during the final data collection when AR was absent. AR performed a preliminary round of data collection on the dancers to familiarize herself and the data collection group with the procedure as well to practice contouring and analyzing the UTC scans using the dedicated computer software. The preliminary scans were not included in the study however they were used for training purposes and all scans were checked by SD for accuracy. SD reviewed each participant's scan and contour for the first round of data collection that was used for studies. For the subsequent four time points, SD reviewed scans at random, and any questionable scans, for accuracy and consistency.



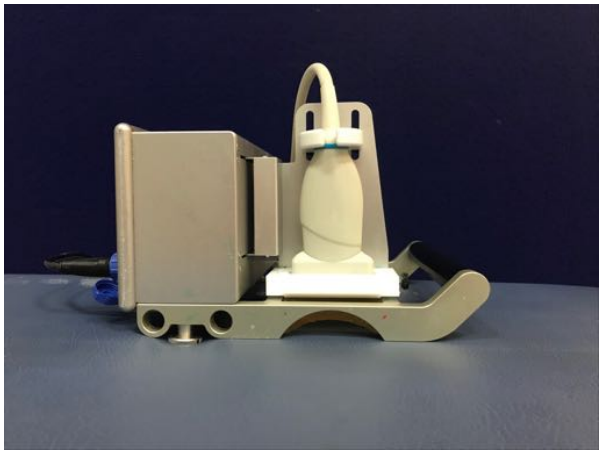


Image 1: Ultrasound tissue characterization tracker and stand-off pad (above)
Image 2: UTC set up for data collection (left)

3.2b Ultrasound tissue characterization: Scanning procedure and contouring parameters

To ensure the patellar tendon was linear in order to optimize imaging, the participants were positioned in supine with their left knee flexed to 90°. The UTC stand-off pad was placed directly over the long-axis of the patellar tendon and alignment was confirmed with real-time imaging. A linear-array ultrasound transducer (SmartProbe 10L5, Terason 2000+; Teratech) was fixed in the tracking device. Once the inferior pole of the patella and the proximal patellar tendon attachment were clearly identified, the transducer moved automatically along the stand-off pad, capturing 600 transverse images, every 0.2mm, as the transducer traveled distally along the tendon.

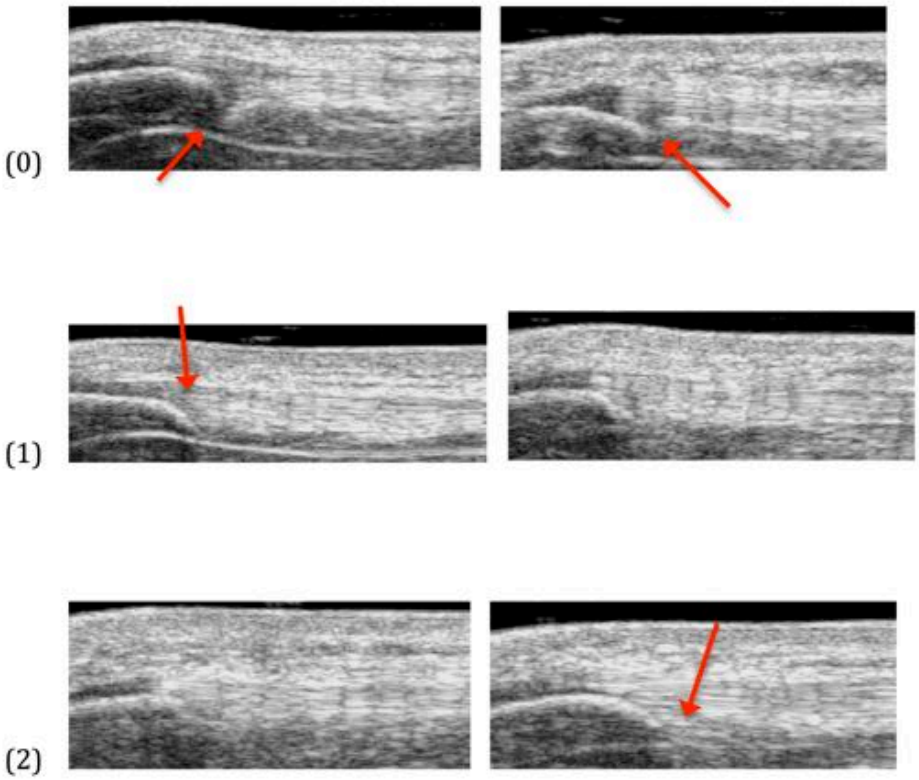
After all scans were completed, all identifying data were removed from the scan to ensure blinding during analysis. The region of interest (ROI) of the patellar tendon was between the inferior pole of the patella and the proximal 2cm of the tendon. In intervals of less than or equal to 4mm along the longitudinal axis, manually selected contours in the transverse were placed to define the ROI within the image. Based on these contours, the dedicated software interpolated the manual contours to automatically generate a 3-dimensional volume of the tendon. From this, the UTC algorithm (UTC2010, UTC Imaging) analyzed the stability of echopattern within the 3-dimensional volume. Stability of echopattern was quantified as a percentage for echo-types I-IV (Section 1.4).

3.2c Greyscale score: motivation, development and reliability

Describing images on greyscale ultrasound is highly subjective and can make studying changes in tendon appearance over time difficult or inaccurate. Ducher, et. al. established a greyscale image-based tool to describe distal patellar tendon attachment appearance throughout puberty [23] and this tool has been used and further developed in other research studies[22-24]. As there is limited research describing the appearance of the proximal patellar tendon attachment during puberty, and because this project is monitoring changes in tendon appearance over time, we developed a tool to describe proximal tendon appearance using an image-based score (Fig 3a-c). This greyscale score requires validation with future research to enable other studies study the proximal patellar tendon appearance.

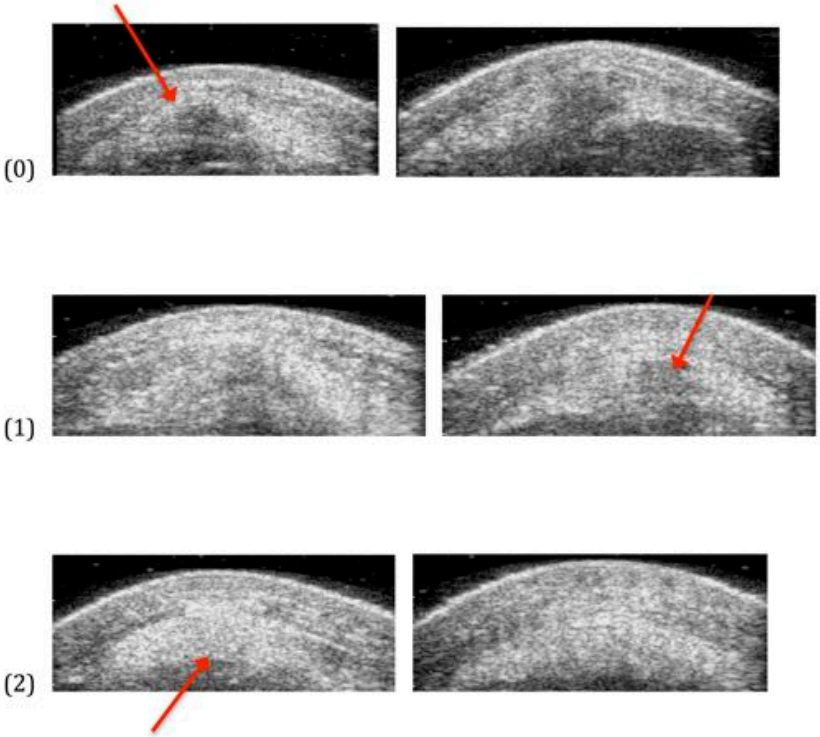
Patterns of variation and different types of proximal patellar tendon appearance on greyscale were consistently seen while performing UTC analysis in the first round of data collection. Participants in this cohort were diverse in pubertal status, some had a mature tendon appearance. The following images were selected to represent the Greyscale Score that was developed based on the varied appearance in the sagittal, transverse and coronal planes:

Figure 3a: Continuity of tendon fibers at inferior pole of patella (sagittal plane):



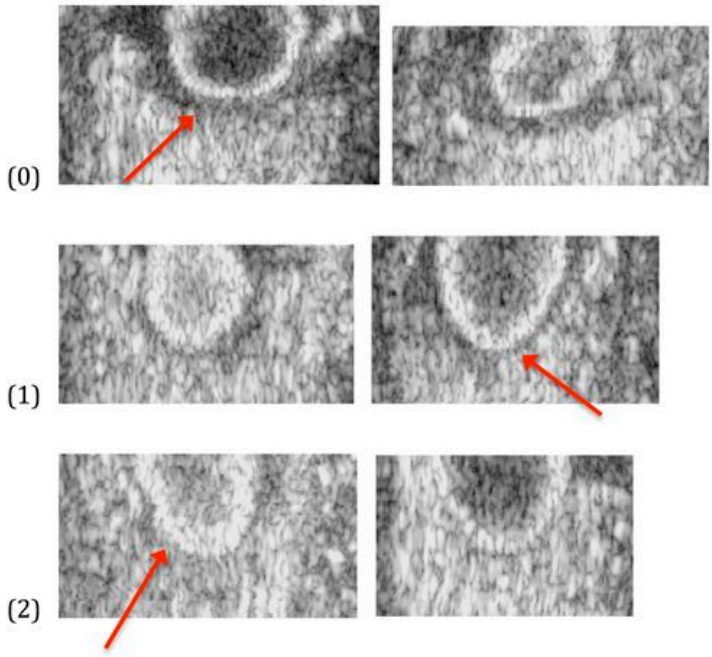
(0) Clear discontinuity
(1) Nearly continuous
(2) Clearly continuous

Figure 3b: Presence of hypoechoic area immediately distal to inferior pole (transverse plane)



(0) Large, dark gray hypoechoic area
(1) Light gray hypoechoic area
(2) No hypoechoic area

Figure 3c. Presence of halo around the caudal half of the patella (frontal plane)



(0) Dark gray halo
(1) Light gray/thin halo
(2) No halo present

To score the greyscale imaged, a number was selected for each category based on most similar ultrasound appearance. The total for each plane was summed and then the total score was categorized: total score of 0-2 was named category 1, total score 3-4 was category 2, and total score 5-6 was category 3. Inter-rater reliability was tested with 30 scans; two raters were blinded to identity or age of the participant. Good reliability was established with a weighted kappa score of 0.617. Studies I and II investigated the relationship between the Greyscale Score and pubertal status.

3.2d Anterior-posterior thickness measurement:

Patellar tendon thickness was measured for studies I and II. Thickness was measured by anterior-posterior diameter in the middle of the patellar tendon in the axial plane using the greyscale images on the UTC. Three measurements were taken; one at the inferior pole of the patella, 1 and 2 cm distal. For study I, the thickness was reported in centimeters (as UTC reports measure in cm) and for study II it was reported in millimeters to be consistent with other literature.

3.2e Maturity offset and peak height velocity

Several methods exist to measure pubertal status in adolescents. Assessing pubertal status can be difficult, as the more accurate methods tend to be more invasive. The gold standard for measuring puberty is Tanner staging [59]. This method involves clinical evaluation of the appearance of secondary sexual characteristics compared to a five-stage sexual maturation scale. This method would be difficult to do when collecting data on many young students at once and faculty at the involved institutions were hesitant to have such a procedure done repeatedly for the longitudinal studies. There are also ethical concerns as well as limitations to accuracy of the self-reported version of Tanner staging. Another commonly used method of measuring skeletal maturity is the FELS method of hand-wrist x-ray [60], though due to logistics and cost constraints, this was not a realistic option for this study. As an alternative, Mirwald, et. al. developed a non-invasive approach to estimate puberty by calculating an adolescent's distance in years to reaching peak height velocity [19]. This distance is called maturity offset and it is a function of age, height, weight, and leg length. As our studies were measuring changes of proximal patellar tendon attachment during adolescence, using maturity offset fitted appropriately with the aims and study design. Additionally, faculty at the schools, parents, and students all were comfortable with this outcome measure.

Measurements were done with the same standardized procedure at every time point. Standing and sitting height were measured in centimeters with a stadiometer. Sitting tall on a table with hips, knees, ankles were flexed to 90° and feet supported, sitting height was measured from the ischial tuberosity to the top of the participant's head. Sitting height was subtracted from height to calculate leg length. The same electric scale was used every time to measure weight in kilograms. Age was calculated using the participant's date of birth and the date of data collection. Maturity offset was calculated using the following two equations:

MALE= =((-9.236)+(0.0002708*(leg length*sitting height))-(0.001663*(age*leg length))+(0.007216*(age*sitting height))+(0.02292*(weight/height)))

FEMALE =((-9.376)+(0.0001882*(leg length*sitting height))+(0.0022*(age*leg length))+(0.005841*age*sitting height))-(0.002658*(age*weight))+(0.07693*(weight/height))

A negative maturity offset is interpreted as the distance (measured in years) before peak height velocity (PHV), which is termed pre-PHV. A positive maturity offset is interpreted as distance after PHV, termed post-PHV. Once maturity offset was calculated, peak height velocity was also calculated by subtracting maturity offset from the participant's age to estimate the age of the peak growth spurt in years (ie: PHV=13.50, meaning the peak growth spurt is estimated to be when the participant is 13 years and 6 months old).

For study I, participants were considered to be pre-PHV if their maturity offset was less than or equal to negative one. They were considered peri-PHV if maturity offset was between -1 and 1. They were considered post-PHV if maturity offset was greater than or equal to one. For studies II and III participants were considered pre-PHV at the start of the study if their maturity offset was negative, and post-PHV at the start of the study if their maturity offset was positive.

3.2f Pain monitoring: VISA-P and single leg decline squat

To monitor pain in study III, participants filled out the Victorian Institute of Sport Assessment for the Patellar Tendon (VISA-P) questionnaire, which indexes the severity of patellar tendinopathy symptoms. The VISA-P is scored out of a possible 100 points; a score above 80 is interpreted as able to function fully in activity while a lower score indicates dysfunction and pain associated with tendinopathy [61]. The VISA-P has excellent test-retest and inter-rater reliability ($r>0.95$) [61], with a minimum clinically important difference (MCID) change in score of 13 or greater or an improvement of 15.4-27% of baseline (depending on baseline score) [62]. Participants also performed the single leg decline squat (SLDS) and reported pain, and location of pain, on a visual analog scale at every time point. The single leg decline squat was performed on a 25° decline board where the participant does a single leg squat to 60° and then reported pain below the patella on a 100mm long visual analog scale, where no pain is 0mm and worst pain ever is 100mm [63]. A 25° decline has been shown to increase the load on the patellar tendon as compared to regular eccentric squat [64] and will therefore provoke symptoms if present.

3.2g Activity monitoring: 1-week and 6-month jumping participation, Likert scale:

An advantage to studying dancers is their low attrition rate and a fairly regular dance schedule. These two factors allowed for easy monitoring of activity loading. To monitor regular dance activity, the participants' teachers reported daily dance class schedule as well as monthly dance class, rehearsal, and performance schedule for all grades participating in the study. The participants answered a questionnaire at each time point describing any time-loss and reasons for days missed in the previous week and 6 months. They also reported on any injuries and modifications they were making in dance class if they were not participating fully (ie: not jumping). To gauge how the previous week's activity compared to the previous 3 months, the students rated it from "very much less activity" to "very much more activity" on a Likert scale of -4 through +4, with 0 being "same activity," -4 being "very much less activity" and +4 being "very much more activity."

3.3 Statistics

3.3a Study I (Non-parametrics)

Analysis for this study was done in SPSS. Chi-square was used to test the Greyscale Score (using the categories) with maturity offset. The Levene's test of homogeneity

was used to test the homogeneity of variance for echo-type composition by maturity offset, and one-way ANOVA was used to test the relationship between maturity offset and echo-type as well as thickness. Significance was set at $p \leq 0.05$.

3.3b Study II (Longitudinal analysis)

Analysis for this longitudinal study was done in the R statistical package using the plm package for longitudinal data. Economics researcher, Dr. Daniel Brent, PhD, consulted on this analysis. As not all the participants were pre-PHV at the start of the study, we aimed to test both the main effect of maturity offset on the outcomes of interest as well as the differential effect of maturity offset on these outcomes after dancers had passed PHV. To do this analysis, we used a random effects multivariate longitudinal data regression model employing an interaction term to test for a differential effect of maturity offset on the outcomes variables after participants reached PHV. The outcomes of interest were: echopattern by type (I-IV) at 1 and 2 cms distal to the proximal attachment, anterior-posterior thickness at the inferior pole, 1 and 2 cms distal, and greyscale score. By using the random effects longitudinal model, analysis of both within and between individual variations of longitudinal data can be measured [65]. Maturity offset was the independent variable and the dependent variables were each of the included outcomes of interest. The interaction term distinguished if maturity offset had a greater differential effect on the outcomes for the participants who had passed peak height velocity at the start of the study. The regression model is as described in the following equation:

$$y_{it} = \alpha + \beta_1 MaturityOff + \beta_2 Maturity\ Off x Peak\ Height\ + \epsilon_{it}$$

y_{it} represents the outcome measures of interest, $MaturityOff$ is the maturity offset measure (in years), $PeakHeight$ is an indicator variable equal to one if the participant was past peak height velocity at baseline (16 participants) and zero if pre-PHV at baseline (36 participants), and, ϵ_{it} represents the idiosyncratic error term. In the equation β_1 represents the main effect of maturity offset on the outcome of interest and β_2 represents the differential effect of maturity offset for the post-PHV participants. The total effect of maturity offset on the outcome variables, therefore, for participants post-PHV is $\beta_1 + \beta_2$. Additionally, to measure the change in greyscale score over time, we regressed the Greyscale Score by time period, the results representing the change between baseline and each time period. Significance was set at $p \leq 0.05$.

3.3c Study III (Non-parametric)

Analysis of descriptive statistics and linear regressions were done using the R statistical package. The Mann-Whitney-Wilcoxon test was used to analyze the differences between normal and abnormal group features. The variables tested included: maturity offset, greyscale score, UTC echopattern, single leg decline squat, VISA-P score, jump participation for the previous week and 6 months, and Likert scale for physical activity. Wilcoxon test compared differences between groups both at baseline and throughout the study. Included in the results are mean and standard deviation for each group as well as p-value for Wilcoxon test. For the UTC echopattern, the effect of maturity offset on echopattern was also tested using the longitudinal analysis described above in study II, however the interaction term was set to equal one if the participants were in the pathology group and zero if they were in the normal group. The analysis was used to determine if the presence of pathology on greyscale had a greater differential effect on the development of disorganized echo-type (III+IV). Significance was determined at $p \leq 0.05$.

4. Results and Discussion

This section will present, discuss and synthesize the most important results of these three studies. The manuscripts at the end of this thesis have a detailed presentation of all results.

4.1 Greyscale Score

In study I there was a positive relationship between the Greyscale Score (by category) and maturity offset (p=0.024). The pre- and peri-PHV participants showed variability in their scores however, both the post-PHV and mature groups trended towards category 3 which was hypothesized to be the most mature tendon appearance (Table 4a).

Table 4a: Greyscale score group incidence by maturity offset category:

Maturity Offset Category (n)	**Greyscale category 1**	**Greyscale category 2**	**Greyscale category 3**
Pre (21)	3 (14%)	11 (52%)	7 (33%)
Peri (27)	0 (0%)	17 (63%)	10 (37%)
Post (9)	1 (11%)	2 (22%)	6 (67%)
Mature (8)	0 (0%)	1 (12.5%)	7 (87.5%)

As study I was a cross sectional study with participants in this cohort in four stages of skeletal maturity, the relationship between the tendon appearance on the Greyscale Score and pubertal status is limited to a small number of participants in each maturity category. There were similarities demonstrated in the appearance of post-PHV participants' tendons and mature participants' tendons. This finding supports the hypothesis that the proximal patellar tendon matures during adolescence and perhaps the variability seen in the pre and peri-PHV participants corresponds with variability of tendon maturation during early and mid adolescence with the proximal tendon becoming mature by late adolescence. This timing would be consistent with the patellar tendon maturation at the distal end, which was seen to form a mature attachment 2 years after PHV [22, 23].

Study II followed the same participants longitudinally and we hypothesized that individuals would progress through the Greyscale Score as they progressed through adolescence, which could validate the score. Maturity offset did not have an effect on the Greyscale score. However, the results of the Greyscale score regression analysis over time showed that between data collection time points one and four, there was a positive progression, meaning that the scores increased relative to baseline. The score progression was not a consistently step-wise progression as the score decreased slightly on the third time period compared to the second (Table 4b). For the final time point there was large decrease in score. The results for the first four time points support our hypothesis that as participants progressed through adolescence and became more skeletally mature, the changes in their tendon appearances were somewhat consistent with the progression proposed in the Greyscale Score. The negative effect seen at the fifth time-point can either be explained by a poor design of the Greyscale Score or possible inter-scanner inconsistency. The fifth time period was the only one that used a different person to perform the UTC scans and possibly affected the consistency of the greyscale images with the

previous time points. While UTC has been shown to have high inter and intra-observer reliability [29-31], it is possible that human inconsistencies and the subjective nature of the score contributed to this result. This significant difference between the fifth time period and all others was not seen in the echopattern analysis.

Table 4b: The Effect of Time on Change in Greyscale Score:

	Greyscale Score
	(s.d.)
Time 2	**0.595****
	(0.238)
Time 3	**0.424****
	(0.213)
Time 4	**1.172*****
	(0.222)
Time 5	**-2.053*****
	(0.231)
N	**216**
R2	**0.559**
Adjusted R2	**0.408**
F Statistic	**50.760*** (df = 4; 160)**

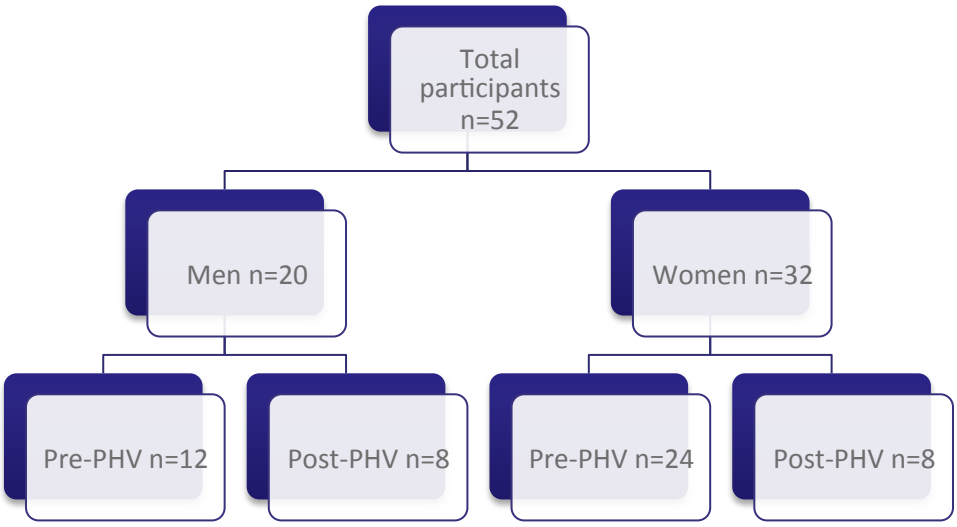
Notes: ***Significant at the 1 percent level.
**Significant at the 5 percent level.

The third study compared the baseline Greyscale Scores of those who went on to develop pathology to those who did not. Using a Wilcoxon test, there was no difference between the two groups at baseline (p=0.11). This finding is suggestive that pathology does not appear to be associated with a specific appearance or category on the Greyscale score. Additionally, the greyscale appearance may not be predictive of, or associated with, future pathology development. The sample size of 57 was small; more research in larger cohorts is needed to clarify this relationship.

4.2 Normal Development

The 52 participants in this cohort included 32 women and 20 men with a baseline age range of 11-18 years. These dance students participated in 10-27 hours of dance per week including technique class and rehearsal, with hours per week increasing with age. The pubertal status of the participants at baseline was: 24 women were pre-PHV and 8 women were post-PHV at baseline, 12 men were pre-PHV and 8 were post-PHV at baseline (Figure 4a).

Figure 4a: Baseline participant demographics:



4.2a Ultrasound Tissue Characterization- Change in Echopattern:

The hypothesis for tendon composition was that maturity offset would have an effect on echopattern. Specifically, we anticipated a change towards more aligned fibrillar structure, echo-type I, in the later stages of puberty (after peak height velocity). We investigated both the main effect of maturity offset on the whole sample as well as the differential effect (interaction effect) of maturity offset on the post-PHV sub-sample.

There was no main effect of maturity offset on any of the echo-type in the first centimeter, however, there was a positive interaction effect on echo-type I (Table 4c). This is interpreted as maturity offset having a differential affect on type I echopattern after PHV is reached. The magnitude of the differential effect was a 4.2% additional increase in echo-type I per year after PHV as compared to the effect of maturity offset for the entire cohort. This means that the total effect of maturity offset on echo-type I in the post-PHV sample was a 3.4% increase per year, calculated by $(\beta_1 + \beta_2)$ or *(0.042 + -0.008) x 100* (Table 4c and Figure 4b).

In the second centimeter there was a main effect of maturity offset on echo-type III with an increase of 0.7% per year, however there is also an equivalent negative interaction effect of maturity offset on echo-type III after PHV. This is interpreted as a slight increase in disorganized echopattern prior to PHV with a reduction in disorganized echopattern after PHV. Additionally, there was a positive interaction effect on echo-type I which is similar to that seen in the first centimeter: the differential increase of type I after PHV is 4.0% however the total effect seen is a 2.1% (equation as above) increase in echo-type I per year after PHV in this sample (Table 4d and Figure 4c).

These results demonstrate that after peak height velocity is reached, maturity offset has an effect on echopattern, increasing the composition of stable echo-type I and reducing disorganized echo-type III. This supports the hypothesis that maturity offset has an effect on tendon composition, though this is seen only after PHV. Additionally, it supports the hypothesis

that adolescence is a critical time for tendon maturation, particularly following PHV as the tendon transitions towards more stable structure (Figures 4b and 4c).

Table 4c: Echopattern in First Centimeter:

	Type I (Green)	Type II (Blue)	Type III (Red)	Type IV (Black)
Maturity Offset	-0.008	0.001	0.004	0.002
	(0.015)	(0.015)	(0.003)	(0.002)
Peak Height	-0.006	-0.008	0.009	0.004
	(0.020)	(0.021)	(0.005)	(0.002)
Interaction Effect	0.042**	-0.032	-0.007	-0.003
	(0.020)	(0.020)	(0.004)	(0.002)
N	222	222	222	222
R2	0.096	0.078	0.049	0.049
Adjusted R2	0.084	0.065	0.036	0.035
F Statistic (df = 3; 218)	7.749***	6.128***	3.744**	3.709**

Notes: ***Significant at the 1 percent level.
**Significant at the 5 percent level.

Table 4d: Echopattern in Second Centimeter:

	Type I (Green)	Type II (Blue)	Type III (Red)	Type IV (Black)
Maturity Offset	-0.019	0.009	0.007***	0.002
	(0.013)	(0.013)	(0.003)	(0.001)
Peak Height	0.027	-0.033	0.003	0.004
	(0.018)	(0.017)	(0.003)	(0.002)
Interaction Effect	0.040**	-0.030	-0.007**	-0.003
	(0.017)	(0.017)	(0.003)	(0.002)
N	222	222	222	222
R2	0.128	0.109	0.077	0.068
Adjusted R2	0.116	0.096	0.064	0.055
F Statistic (df = 3; 218)	10.473***	8.825***	6.031***	5.284***

Notes: ***Significant at the 1 percent level.
**Significant at the 5 percent level.

Figure 4b: Change in Echopattern by Maturity Offset in First Centimeter:

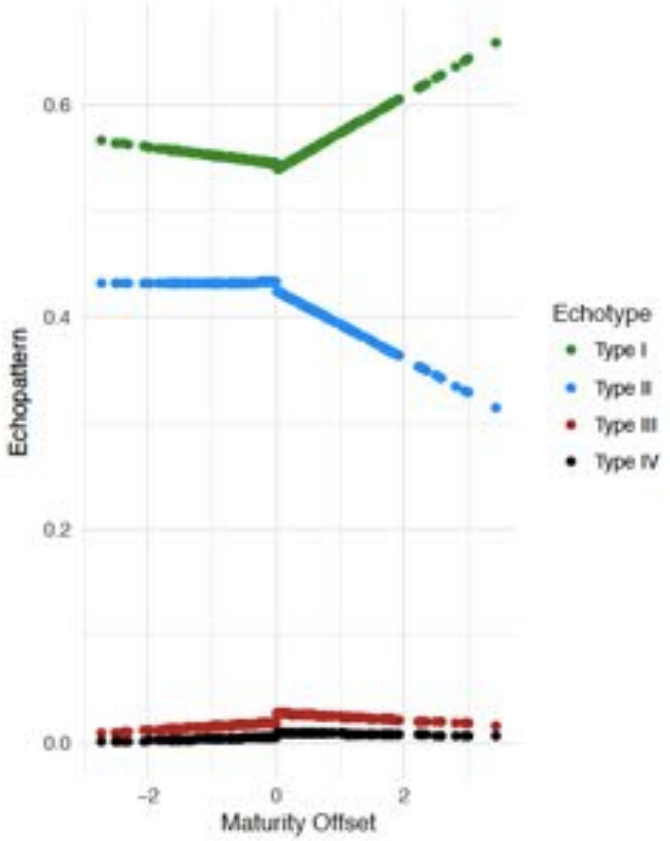
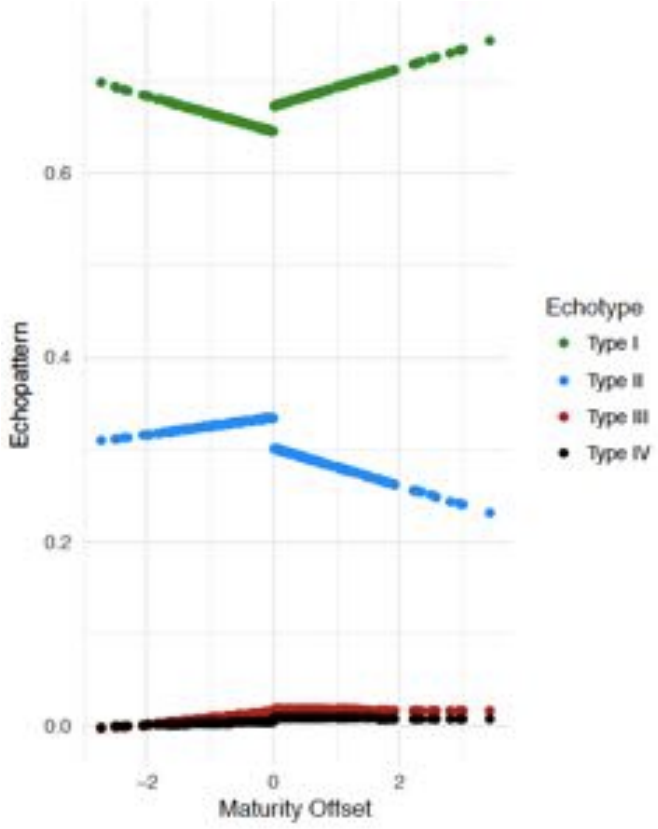


Figure 4c: Change in Echopattern by Maturity Offset in Second Centimeter:



4.2b Change in Anterior-Posterior Thickness:

Increasing patellar tendon thickness with skeletal maturity has been demonstrated in a previous study [20] and we hypothesized similar findings in this study. Using the interaction effect model, there was a main effect of maturity offset on diameter in the second centimeter showing an increase of 0.2mm per year. All of the values for the interaction term, Peak Height, which represents the discreet moment of reaching peak height velocity, showed an increase in tendon thickness. We have included this interaction term because of the relationship it demonstrates between the peak of adolescent growth spurt and a transient adaptation of the tendon to thicken in response to reaching PHV. While this model does not pick up the exact moment of reaching PHV, the significant increase in tendon thickness for the interaction term demonstrates that at baseline the post-PHV participants had thicker tendons than the whole sample (Table 4e).

Table 4e: Anterior-Posterior Diameter:

	AP Diameter (in mm)		
	Inferior Pole	1cm Distal	2cm Distal
Maturity Offset	0.010	0.183	0.199**
	(0.091)	(0.103)	(0.098)
Peak Height	0.280**	0.364***	0.294**
	(0.126)	(0.138)	(0.129)
Interaction Effect	0.009	-0.233	-0.076
	(0.118)	(0.135)	(0.130)
N	220	220	220
R2	0.133	0.174	0.209
Adjusted R2	0.121	0.162	0.198
F Statistic (df = 3; 216)	10.679***	15.076***	19.034***

Notes: ***Significant at the 1 percent level.
**Significant at the 5 percent level.

4.3 Pathology Development

Throughout this 2-year study, 5 of 57 participants (9%) developed hypoechoic areas on their proximal patellar tendons on two or more time periods. None of these five participants had pathology on their tendons at baseline. This group will be referred to as the pathology group. The other 52 participants will be termed the normal group. The normal group was described above in the "Normal Development" section. In the pathology group, three were men and two were women. They ranged from 12-18 years old at baseline. At the start of the study, two were already post-PHV. Of the three who started pre-PHV, one passed PHV before the fourth time period, another before the fifth time period and the third remained pre-PHV throughout the study.

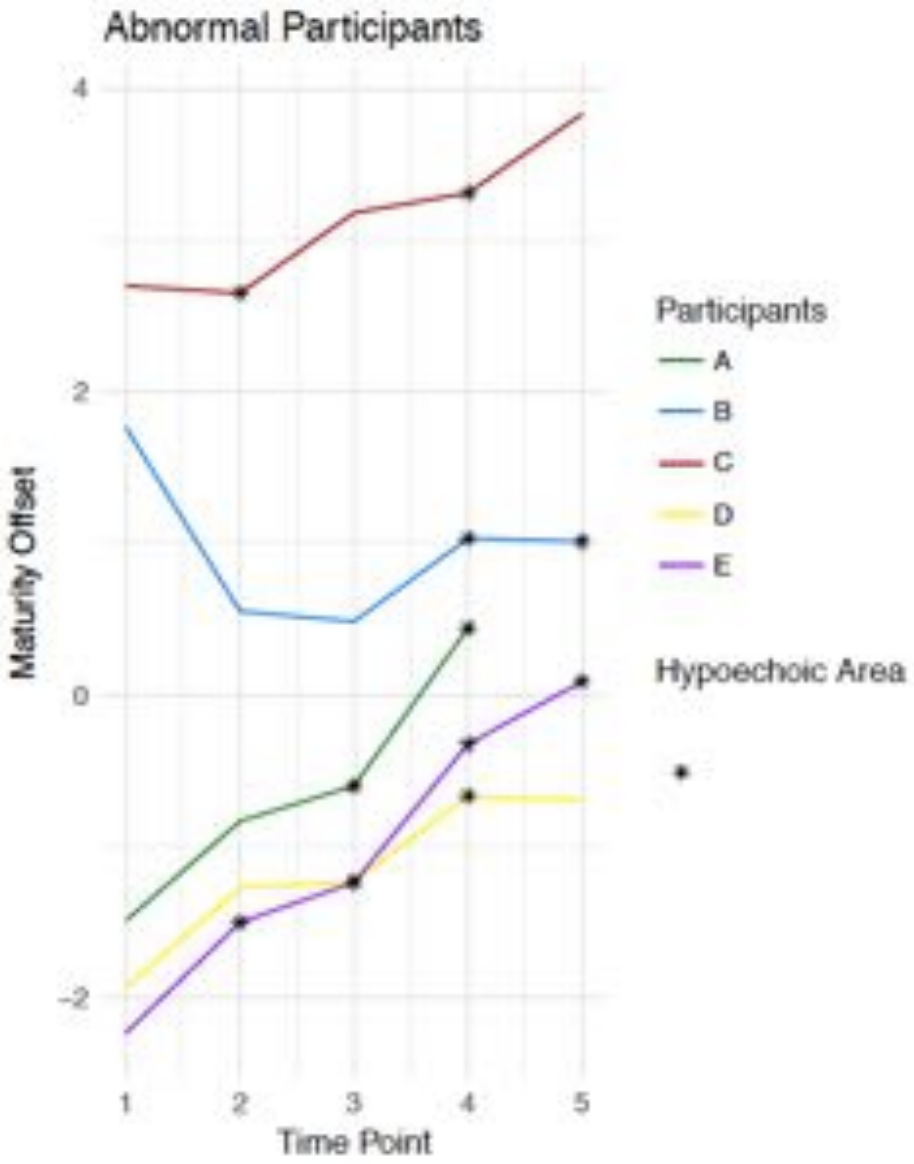
Table 4f: Comparison of Normal and Pathology Participants: Note: * indicates p< 0.05 for Wilcoxon test

	Start of Study					Throughout Study				
	NORMAL		ABNORMAL			NORMAL		ABNORMAL		
Outcome:	Mean	SD	Mean	SD	Wilcoxon	Mean	SD	Mean	SD	Wilcoxon
Maturity Offset	-0.38	1.33	-0.24	2.11	0.79	0.09	1.18	0.29	1.76	0.97
Echotype I	0.63	0.12	0.68	0.11	0.59	0.62	0.10	0.63	0.10	0.18
Echotype II	0.36	0.11	0.30	0.10	0.37	0.35	0.10	0.33	0.08	0.47
Echotype III	0.01	0.01	0.01	0.01	0.29	0.02	0.02	0.03	0.04	0.00*
Echotype IV	0.00	0.00	0.01	0.01	0.33	0.01	0.01	0.01	0.02	0.01*
Greyscale Score	3.87	1.56	2.40	2.30	0.11	3.94	1.72	X	X	X
VISA-P	96.14	10.26	80.00	44.72	0.87	94.45	10.95	90.17	22.04	0.74
SLDS	4.54	11.18	0.00	0.00	0.33	4.79	12.99	11.42	19.03	0.05*
Jump-week	0.08	0.27	0.00	0.00	0.54	0.14	0.35	0.20	0.41	0.44
Jump-6mo	0.10	0.30	0.20	0.45	0.49	0.27	0.44	0.28	0.46	0.88
Likert scale	-0.49	1.44	0.20	1.48	0.28	-0.35	1.37	-0.09	1.78	0.25

4.3a Maturity Offset/Peak Height Velocity and Pathology development

There was no difference in maturity offset either at baseline or throughout the study between the normal and pathology groups (Wilcoxon test p=0.79 and p=0.97 respectively, Table 4f). Within both groups there was a distribution of participants throughout the maturity offset spectrum. In the normal group, 10 of 52 participants were still pre-PHV at the end of the study, 9 or whom were women. In the normal group, 16 were post-PHV throughout the entire study. As there were still several participants who had not reached PHV by the end of the study, there may be more cases of pathology that develop for those participants prior to tendon maturation and because we only analyzed the left tendon, there is a possibility that more pathology developed in the right leg tendons. However, because we did not see a difference in the maturity offset of the two groups, and because the pathology group developed the pathology at different stages of puberty (Figure 4d), we cannot yet link the timing of pathology development directly with the timing of peak height velocity. These findings are consistent with the cross sectional study (study I) that found a high degree of tendon variability in structure and appearance during pre and peri PHV stages. This demonstrates that the timing of normal tendon development is highly variable around PHV and the same was found for the timing of pathological tendon development. Interestingly, the participant who presented with the most occurrences of pathology on the tendon (abnormal at 4 time points) was also the least skeletally mature participant in the pathology group at the start of the study. The most skeletally mature participant appeared to have only transient hypoechoic areas on the tendon (Figure 4d). However the sample size was small (n=5) and the participants were all at different stages of growth throughout this study.

Figure 4d: Pathology participants' maturity offset progression with overlay of time-points showing hypoechoic areas:



4.3b Ultrasound Tissue Characterization

At the start of the study, there was no difference in the structural composition of echopattern between the normal and the pathology groups (Wilcoxon test for echo-type I-IV p=0.59, p=0.37, p=0.29, and p=0.33 respectively). As the study continued, the pathology group developed an increase of echo-type III and IV disorganized echopattern (Wilcoxon test: p=0.001 for type III and p=0.01 for type IV). In order to determine the magnitude of change in disorganized echopattern, the interaction effect model was used with the combined disorganized echopattern (echo-type III+IV) for the entire cohort and the differential effect on the pathology group (Table 4g). There was a main effect for everyone of an increase of 4.1% for echo-types III+IV combined, and an additional 13.6% increase towards the end of the study in the pathology group. It is worth noting that this effect was not seen in the normal group in study II because all four echo-types were tested separately in study II whereas in study III, echo-types III and IV were combined to measure disorganized echopattern. The timing of the increase in disorganized echopattern is correlated with the timing of the most hypoechoic areas (the fourth time period)

and indicates that hypoechoic appearance on greyscale is also consistent with disorganized echotype on UTC.

Table 4g: Disorganized Echopattern (Type III+IV) Changes Over Time in Normal and Pathology Groups:

Time Period:		Main Effect of Time on Echotype III+IV for All Participants:	Differential Effect of Time on Echotype III+IV for Abnormal Participants:
2		-0.0002	0.001
	sd	-0.006	-0.018
3		0.003	0.003
	sd	-0.005	-0.017
4		0.041***	0.136***
	sd	-0.005	-0.017
5		0.036***	0.050**
	sd	-0.005	-0.02

Notes: ***Significant at the 1 percent level.
**Significant at the 5 percent level.

4.3c Pain

Pain and dysfunction (VISA-P) were assessed in this study and there were no differences in scores for the two groups at the start or throughout the duration of the study (Wilcoxon test p=0.87 at the start and p=0.74 throughout). The normal group had a mean and median VISA-P score of 96.1 (mean SD=10.3) at the start of the study and a mean of 94.5 (SD =11.0) throughout the study, both of which are considered fully functional for sports. The median score for the normal group ranged from 93 to 96.1 at each time point (Table 4h). The prevalence of participants who scored below 80 at each time point in the normal group was between 4-14%. In the pathology group, there were also low reports of pain with the baseline VISA-P score mean at 80.0 (SD 44.7) and a median between 80 and 98.6 at each time point. A score below 80 is considered symptomatic. Throughout the study this group averaged 90.17 which is not considered symptomatic. The pathology group had much higher standard deviations than the normal group, indicating more variability in their symptoms (Table 4h).

There were no differences in baseline pain on the single leg decline squat between the normal and pathology groups (Wilcoxon test p=0.33), however as the study progressed, there were differences in pain reports between groups that were very close to significance (p=0.055). The normal group reported a mean pain score of 4.8mm (SD = 13.0) and a median score between 2.4 and 6.5 at each time point. The pathology group reported a mean score of 11.4mm (SD=19.0) throughout the study, with a median score between 0 and 20 at each time point (Table V). The reports of pain for the pathology group were still relatively low and only two of five pathology participants reported pain at all; the same two participants had changes on both the

SLDS and the VISA-P. The other pathology group participants did not report pain, which provides further evidence that pathology may not cause pain.

Other studies have shown that the presence of pathology does not necessarily correspond with painful symptoms [2, 53] and people with pathology within their tendons may also have sufficient healthy tendon tissue as well [54], which would reduce the risk of pain and dysfunction. This study does reinforce the findings that pathology is a risk factor as opposed to a sign of dysfunction. The fact that pathology develops in adolescence and may remain within the adult tendon, increasing the risk of developing symptoms, means that more research is needed to develop prevention strategies focusing on the adolescent population to reduce initial pathology development.

Table 4h: Pain reporting on VISA-P and SLDS:

	TIME PERIOD:				
	1	**2**	**3**	**4**	**5**
Test and Group:					
# Participants with VISA-P under 80 in Normal group (n=52)	4	5	7	5	2
# Participants with VISA-P under 80 in Pathological (n=5)	1	0	1	0	1
Median VISA-P Score Normal Group	96.14	93.64	94.38	93	95
Median VISA-P Score Pathology Group	80	93	92	98.6	86.5
Median SLDS Normal Group	4.5	4.6	6.5	5.6	2.4
Median SLDS Pathology Group	0	15.6	19.4	3.8	20

4.3d Load Volume

Overload is one of the leading extrinsic risk factors for developing symptoms [56] and it was hypothesized that there would be a difference in the loading volume of the normal and pathology groups during this study. The results, however, demonstrated no difference in jumping during class participation for the previous week, 6 months, or on the Likert scale for the two groups ($p=0.44$, $p=0.88$ and $p=0.28$ respectively). Perhaps loading volume and overload has more to do with the onset of symptoms alone versus the initial development of pathology. If this is the case, it is supported by the pain reports, which show little differences between the normal and pathology groups. If overload is a key risk factor to developing symptoms and reports of overload were not seen in the pathology group compared to the normal group, it would be consistent with the low reports of pain in three out of five pathology participants.

5. Limitations

Using maturity offset to estimate puberty or adolescent growth spurt was a limitation of this study. Dancers have a tendency of having low body fat, and delayed menarche in women, which could have affected the accuracy of the equation in this population. We chose to use elite ballet dancers because of their high jumping load and low attrition rate however they do have a lower incidence of patellar tendinopathy that other high jumping sports like basketball and volleyball, as those adolescent athletes can be more difficult to follow over such a long time. Another limitation was the heterogeneity of skeletal maturity throughout the study. Because some participants were already post-PHV at the start of the study, we had to modify our analysis to see if maturity offset affects tendon changes differently before or after peak height velocity though it would have been more optimal to watch the whole cohort start pre-PHV and follow through until 2 years after PHV. A range of maturity at baseline was chosen as this is the first study to investigate this tendon during adolescence and a cohort over all maturity groups was important.

While the UTC was selected because of it's ability to detect subtle changes overtime, it is still a fairly novel device with limited criterion validation in human tendons. While this validation would difficult to produce, it limits the generalizability of the results. Another limitation was having a different scanner perform the UTC data collection on the last time-period. While this was ultimately an unavoidable substitution, ideally the same scanner would have performed all of the data collection.

6. Conclusions

This 2-year longitudinal project observed and quantified the proximal patellar tendon maturation in adolescent elite ballet dancers. We observed a high degree of variability in tendon appearance and echo-type structure leading up to peak height velocity that became more organized and consistent with mature appearance approximately a year after peak height velocity. The evidence presented in this study supports the hypothesis that the proximal patellar tendon becomes mature during adolescence, specifically late adolescence in the sample observed.

A more detailed investigation into tendon changes during this period with an image-based Greyscale score depicts the variety in tendon appearances during adolescence. The Greyscale score, which has high inter-rater reliability, allows further research to have a basis for describing tendon appearance. Future research should modify the scale appropriately to validate it with maturity status.

Throughout this study, we observed the development of pathology in the proximal tendons of 9% of the participants. No study has monitored and demonstrated the development of proximal patellar tendon pathology in adolescent athletes before, and this clarifies the timing of pathology onset that can occur during adolescence. The findings in this study showing 9% of elite adolescent ballet dancers developing pathology is similar to that seen in the adult elite ballet population of 12% [2]. As not all of the participants reached PHV by the end of the study, it is possible that more will develop tendon abnormalities before they form mature tendons. Because the prevalence seen to develop in adolescence was so similar to the adult population, adolescence is a crucial time for both normal and pathological tendon formation.

Tendon pathology was seen to develop in pre- and post-PHV participants and was only associated with pain in two out of five pathology participants. The relationship between structure and symptoms continues to be investigated in other research studies, however these findings support the idea that pathology is not always associated with pain but it may be a

precursor to pain and is a key risk factor. There was a correlation in the timing of increased hypoechoic areas on greyscale and increased disorganized echo-type III+IV on UTC. This finding suggests that there is a relationship between greyscale hypoechoic areas and UTC structural disorganization. This may be helpful as UTC is not widely available for research or clinical purposes.

7. Perspectives

The main two findings in this thesis: normal proximal patellar tendon maturation occurs during adolescence and pathology is a disease process of adolescence, have not been demonstrated previously in the literature. The timing of proximal patellar tendon maturation is similar to that seen in the distal patellar tendon, which occurred approximately 2 years after PHV [22, 23]. With 12% of elite adult ballet dancers demonstrating pathology on their proximal patellar tendons [2], the incidence in this sample was similar at 9%, providing evidence that this is a disease process of adolescence. Other sports show even higher prevalence of pathology in the adult population, such as 32% of elite basketball players [11], which may have implications for the prevalence and development in adolescence. To date, no longitudinal studies have observed early and mid adolescent basketball players to identify this relationship.

As Ultrasound Tissue Characterization is used more in international clinical research, this thesis project adds the growing body of evidence utilizing this tool and establishing baseline data for future projects. It provides an image-based greyscale score to describe the adolescent patellar tendon appearance in three-planes. It also establishes baseline data for normal patellar tendon tissue structure during adolescence and begins to describe pathological changes reflected on both greyscale and by echopattern. Future research can build on this study to add depth and dimension to our understanding of tendon development.

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Patellar Tendon Development in Adolescent Dancers

This declaration concerns the following article:
Characterising the proximal patellar tendon attachment and its relationship to skeletal maturity in adolescent ballet dancers

The PhD student's contribution to the article: *(please use the scale (A,B,C) below as benchmark*)*	(A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable by experiments	B
2. Planning of the experiments and methodology design, including selection of methods and method development	B
3. Involvement in the experimental work	C
4. Presentation, interpretation and discussion in a journal article format of obtained data	C

**Benchmark scale of the PhD student's contribution to the article*		
A. refers to:	*Has contributed to the co-operation*	*0-33 %*
B. refers to:	*Has contributed considerably to the co-operation*	*34-66 %*
C. refers to:	*Has predominantly executed the work independently*	*67-100 %*

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Patellar Tendon Development in Adolescent Dancers

This declaration concerns the following article:

Proximal patellar tendon pathology can develop during adolescence in young ballet dancers: a 2 year longitudinal study.

Proximal Patellar tendon pathology can develop during adolescence in young ballet Dancers: a 2 year longitudinal study

The PhD student's contribution to the article: *(please use the scale (A,B,C) below as benchmark*)*	(A,B,C)
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2. Planning of the experiments and methodology design, including selection of methods and method development	C
3. Involvement in the experimental work	C
4. Presentation, interpretation and discussion in a journal article format of obtained data	C

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Characterising the proximal patellar tendon attachment and its relationship to skeletal maturity in adolescent ballet dancers

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Summary

***Background*: It is unknown how and when the proximal attachment of the patellar tendon matures; puberty may be key in ensuring normal tendon formation. The aim of this study was to investigate the features of the proximal patellar tendon attachment at different stages of skeletal maturity, to help gain an understanding of how and when the tendon attachment matures.**
***Methods*: Sixty adolescent elite ballet students (ages 11-18) and eight mature adults participated. Peak height velocity (PHV) estimated skeletal maturity. Ultrasound tissue characterisation (UTC) scan was taken of the left knee and analysed for stability of echopattern. An image-based grading scale for greyscale ultrasound was developed to describe the tendon appearance. Anterior-posterior thickness was measured at the inferior pole of the patella, 1 and 2 centimetres distally. Outcomes were compared with skeletal maturity.**
***Results*: Mid-portion patellar tendon thickness increased with skeletal maturity (p=0.001 at 1cm and p=0.007 at 2 cm). There was more variance in structural appearance (greyscale classification and UTC echopattern) in pre and peri-PHV participants. Tendon attachment one-year post PHV appeared similar to mature tendons.**
***Conclusions*: Early adolescence was associated with highly variable tendon appearance, whereas the tendon appeared mature after PHV. Adolescence may be a critical time for the formation of normal tendon attachment.**
Level of evidence: IIb individual cohort study.

KEY WORDS: ballet students, jumper's knee, jumping athletes, patellar tendon development, skeletal maturity, ultrasound tissue characterisation.

Introduction

Patellar tendinopathy, also known as "jumper's knee", is the clinical condition of pain and dysfunction, predominantly at the attachment of the patellar tendon to the patella that is associated with jumping and sports with explosive movements. The relationship between jumper's knee symptoms and tendon pathology seen on imaging is complex; of those who present clinically with pain, 79% have patellar tendon pathology on ultrasound imaging (fusiform swelling and/or a hypoechoic region)[1]. The presence of pathology on imaging is one of several risk factors for pain and dysfunction, yet there is no direct relationship between pathology and the development of symptoms[2].
While changes on imaging are considered a risk factor for developing symptoms, the aetiology of tendon pathology is not known. Pathological changes of the patellar tendon on imaging are seen in adults, but it is unknown if the pathology developed close to the onset of symptoms or if the pathology developed during adolescence. Interestingly, the proportion of adolescent basketball players (aged 14-18 years old) with pathological patellar tendons is similar to that in the adult population (26% in adolescents compared to ~30% in adults)[1]. Furthermore, athletes without pathological changes by the age of 16 have a low risk of developing jumper's knee in adulthood[3]. These data suggest that adolescence may be critical in the development of patellar tendon pathology.
Most of what is understood about patellar tendon maturation is based on general tendon development. Tendons elongate and thicken during postnatal development at a rate proportional to the growth of their

associated muscles and bones[4-6]. By the time adolescents reach approximately 17 years old, the collagen in the tendon matrix is relatively stable[7], which is typically after a person has passed their peak height velocity[8]. Several studies have investigated how the patellar tendon matures; however knowledge is currently limited to the tibial attachment[9-13] with very few investigations at the patella attachment. The appearance of the proximal patellar tendon attachment throughout skeletal maturity and growth is currently unknown.

The aim of this study was to compare the varied appearances of the proximal patellar tendon attachment in young jumping athletes (ballet dancers) across different stages of skeletal development, which was estimated by peak height velocity. The proximal patellar tendon attachment was assessed using Ultrasound Tissue Characterisation (UTC), which allowed for the grey-scale ultrasound appearance to be described visually as well as quantified by the echopattern. As jumper's knee affects primarily jumping athletes, due to high energy storage within the tendon, this study only looked at ballet dancers. This is a preliminary study to determine whether the appearance and UTC echopattern of the proximal patellar tendon attachment differs between various stages of skeletal development. Our hypothesis was that the proximal patellar tendon attachment would transition to a mature appearance during peak height velocity.

Materials and methods

Ballet students from the Australian Ballet School and Victorian College of the Arts Secondary School between the ages of 11 and 18 participated in this study. All participants were regularly involved in daily dance classes. Anthropometric measurements including height (cm), weight (kg) and sitting height (cm) were collected. For sitting height, participants were sitting tall on a table, thighs fully supported on the table and feet supported so knees were comfortably flexed to 90°. The measurement of sitting height was taken from the ischial tuberosity to the top of the participant's head. Leg length was calculated by subtracting sitting height from standing height. Using leg length, height, weight and age, maturity offset was calculated to estimate peak height velocity based on the equation by Mirwald et al.[8].

Eight healthy participants between 21-40 years old who had their patellar tendons imaged previously for other studies were also included. No anthropometric data were available for this group, however because of their ages, they will be assumed to be skeletally mature as the average age to reach skeletal maturity in girls is 14.9 and 15.4 in boys[14].

Participants were categorised into three groups based on the number of years before or after peak height velocity (maturity offset). Participants who were greater than or equal to a year before reaching PHV estimate were categorised as pre-PHV, between one year before and one year after PHV estimate were considered peri-PHV and those greater than or equal to one year after PHV estimate were labeled post-PHV. The older group (21-40) was considered skeletally mature. The Monash University ethics committee approved this study and all participants, or participants' parents if aged under 16 years, gave informed consent. This study meets the ethical standards of the Journal[15].

Ultrasound Tissue Characterisation

UTC is a validated measure with a high degree of inter and intra-rater reproducibility[16]. The UTC scans were taken using a standardised protocol described below: this protocol was adapted from the protocol used for the Achilles tendon[16, 17]. The UTC device has a standardised transducer tilt angle that provides a 3-dimensional image of the entire tendon. The image is analysed based on a computerised algorithm that has also been validated against equine tendon tissue samples[18-20].

One investigator (AR) performed all UTC scans using the standardised protocol on each participants' left knee. Participants were in a supine position with the left knee flexed to 90° in order to position the UTC tracker perpendicular to the patellar tendon. The decision to analyse only the left leg was an *a priori* decision. While ballet dancers have dominant leg preferences most exercises and movements are performed equally on the right and left legs. For the mature participants, the patellar tendon without pathology and pain was selected and scanned and analysed using the same procedure. A linear-array ultrasound transducer (SmartProbe 10L5, Terason 2000+; Teratech) mounted in a tracking device with a motor-drive and built-in acoustic-coupling stand-off pad (UTC Tracker, UTC Imaging) was placed directly on the patellar tendon such that the ultrasound head was perpendicular to the tendon at the patellar insertion. Once a clear image of the tendon and the inferior pole was apparent and the alignment of the standoff pad was verified visually, the transducer was automatically moved distally over the length of the tendon capturing a transverse greyscale image every 0.2 mm over 12 cm. This procedure is repeated in the same standardised manner for all participants.

UTC captures 600 contiguous transverse ultrasound images and renders a three-dimensional greyscale image[20]. Based on the captured images, dedicated software analyses the stability of the echopattern across multiple transverse images categorising the tendon into four echo-types (UTC2010, UTC Imaging), with echo-type I being the most stable and echo-type IV being the least stable[20]. Echo-type I, II, III and IV are represented as green, blue, red and black pixels respectively, where echo-type I and II are considered aligned fibrilar structure and echo-type III and IV represent disorganised structure[16,20,21]. Histopathological specimens from the horse have been correlated with UTC echopatterns to show it is a valid modality to estimate pathology[20].

The region of interest (ROI) for the patellar tendon was selected from the disappearance of the inferior

pole of the patella, extending two centimetres distally. Manually selected contours were placed along the length of the tendon in intervals no more than 4 mm. The UTC software automatically interpolated contiguous ROIs creating a tendon volume where the proportions of each echo-type were calculated. Quantification of the UTC echopattern was performed by the same investigator (AR) blinded to participant identity and age, with the window size set at 25 (slice-thickness 4.8 mm). All contours were reviewed by another trained blinded researcher (SD) to ensure consistency.

Subjective maturation grading

A classification system was developed to categorise the varied appearances of tendon attachments on greyscale imaging. The categories were based on greyscale imaging features in the sagittal (Fig. 1), transverse (Fig. 2) and coronal (Fig. 3) planes. The figures below show the criteria for each score and the total was summed for a total score, then assigned to a "greyscale category." Inter-rater reliability testing was done between two researchers (AR and SD) where 30 UTC scans, selected at random, were independently scored by each rater who was blind to all other analysis.

In the sagittal plane, the tendon was graded a 0 if there was a clear discontinuity between the inferior pole of the patella and the tendon, a 1 if there was some hypoechoic, hyperechoic or normoechoic tissue, but not continuous between the inferior pole and tendon and a 2 if there was a clear normoechoic continuation of the tendon over the inferior pole of the patella (Fig. 1).

In the transverse plane as the inferior pole disappeared, grade 0 was assigned for the presence of a hypoechoic region with well-defined borders. It is hypothesised that these regions are cartilage and are not pathology due to the lack of red and black echotypes on the rendered UTC image. Grade 1 was for images with some light grey or variable echogenic areas without clear borders and grade 2 was for a normal echogenic tendon with no areas of hypoechogenecity (Fig. 2).

In the coronal plane researchers graded the images based on the presence or absence of a dark halo around the lower half of the patella. If there was a dark and/or thick halo around the inferior half of the patella it was graded 0, if there was a thin, light grey or partial halo around the inferior half of the patella it was graded 1 and if there was no halo it was graded 2 (Fig. 3).

Statistical analysis

Participants were excluded if they had pathology in their tendons. Pathology within their tendon was defined as the presence of a hypoechoic area on greyscale US, focal thickening of the tendon and/or thickness greater than 7 mm[22]. For the greyscale

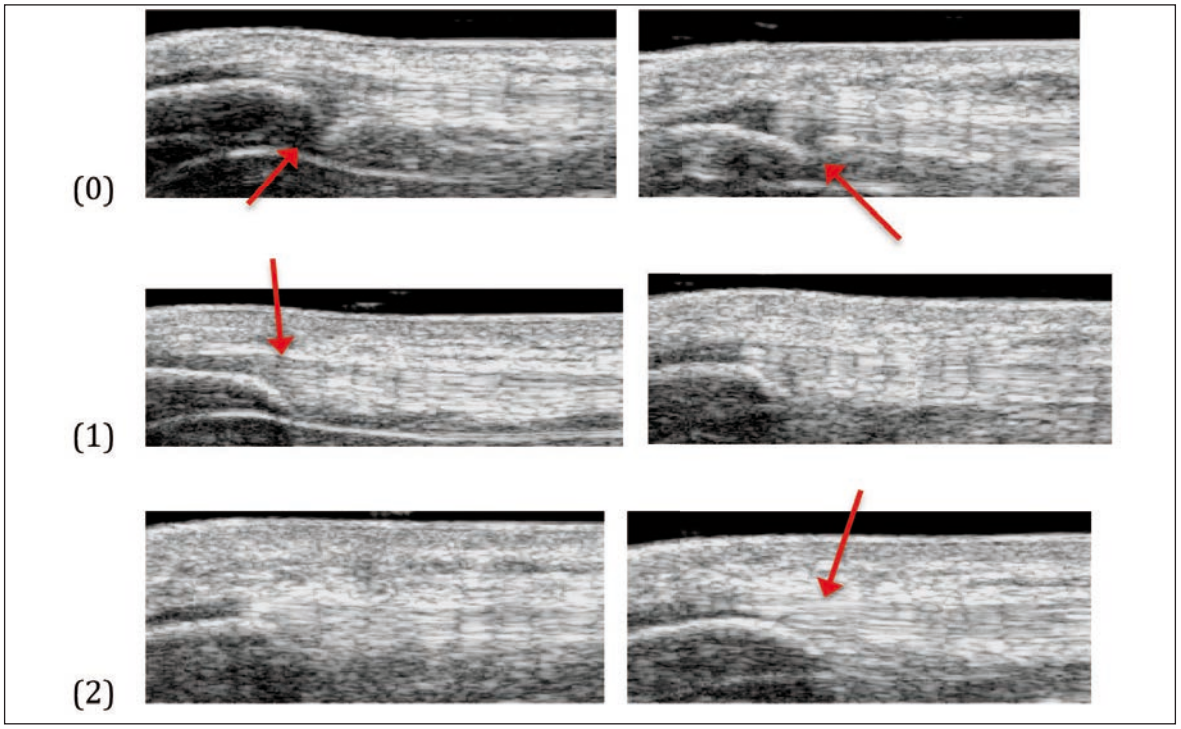

Figure 1. Continuity of tendon fibres at inferior pole of patella (sagittal plane).
(0) Clear discontinuity (between inferior pole of patella and tendon)
(1) Nearly continuous (inconsistent or light grey between inferior pole of the patella and tendon)
(2) Clearly continuous (consistent between patella and tendon)

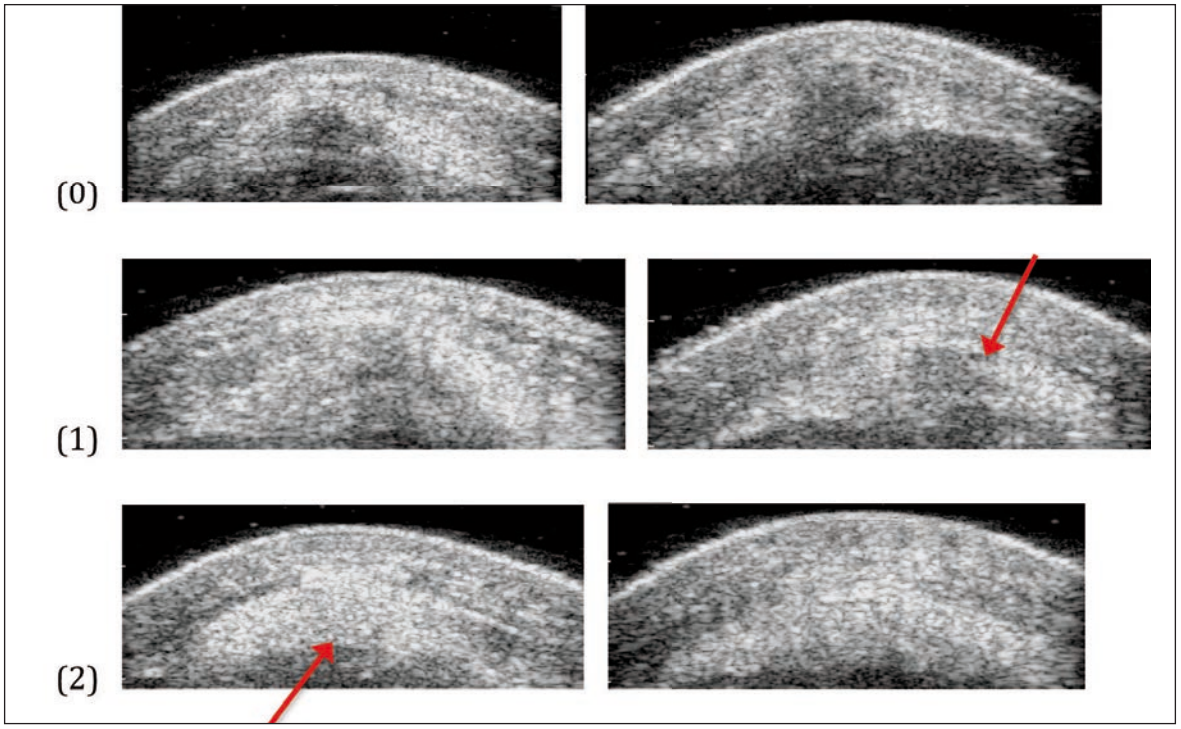

Figure 2. Presence of hypoechoic area immediately distal to inferior pole (transverse plane).
(0) Large, dark grey hypoechoic area.
(1) Light grey hypoechoic/variable echogenic area.
(2) No hypoechoic area.

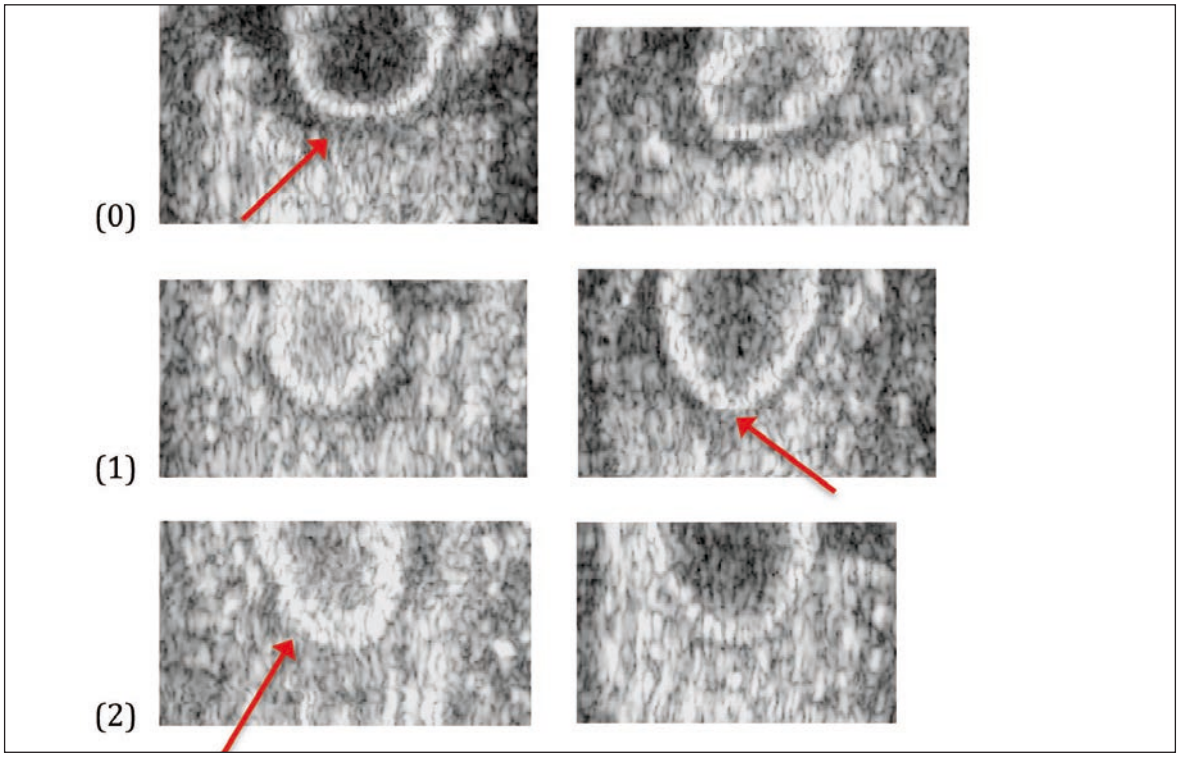

Figure 3. Presence of halo around the inferior half of the patella (coronal plane).
0. Dark grey halo (around inferior half of patella).
1. Light grey/thin halo (may be thin or partial halo on center or sides).
2. No halo present.

classification, the totals of each of the three categories were summed (named the patellar attachment score) and placed into three groups: patellar attachment score of 0-2 was classified as category 1, score of 3-4 was classified as category 2 and score of 5-6 was labeled category 3. Chi-square testing analysed the arbitrary groups with the maturity offset groups.
Levene's test for homogeneity of variance and one-way ANOVA was used to compare maturity offset with the UTC echopattern for the contoured region of interest. One-way ANOVA was also used to compare maturity offset with anterior-posterior thickness across the patella, at the inferior pole, 1 cm and 2 cm distal from the inferior pole.

Results

Of the 60 participants (35 women and 25 men), 23 were pre-PHV, 28 peri-PHV, 9 post-PHV and 8 were mature (Tab. I). After UTC scans were reviewed for quality, where 3 scans were excluded due to poor imaging quality, 65 tendon scans were included; 57 from the ballet students, 8 from the mature group. No pathology was observed in any of the scans.

Anterior-posterior (AP) thickness

The AP thickness increased by group as PHV increased at 1 cm and 2 cm from the inferior pole of the patella (p=.001 and p=.007 respectively). However, the AP thickness at the inferior pole of the patella did not differ significantly between the maturity groups (p=.132) (Tab. I).

Greyscale scoring and grouping

Inter-rater reliability demonstrated good agreement with a weighted kappa score of 0.617. The arbitrary grouping increased with skeletal maturity (p=.024, Tab. II). Within the lower maturity offset groups (pre and peri-PHV) there was a distribution of greyscale scores whereas in the higher maturity offset category (post-PHV) and the mature group most participants were in greyscale category three.

UTC echopattern

There were no significant differences in echo-type across the maturity groups (Tab. III). However, in the pre-PHV and peri-PHV groups there was a greater amount of variability in the echopattern, the variance reduced significantly (p=0.028) in the post and mature groups for the percentage of echo-type I (Fig. 4). Further, there was a trend towards increasing percentage of echo-type I and decreasing percentage of echo-type II seen across skeletal maturity (Tab. III).

Discussion

This study investigated the varied appearances of the proximal patellar tendon attachment during adolescence to gain further understanding of how and when the proximal patellar tendon matures. We hypothesised that the tendon would transition to a mature attachment during this developmental stage. This study identified four main findings: 1) the tendon does not change significantly in thickness directly at the inferior pole, yet increases in thickness with maturity closer to the midsubstance of the tendon; 2) there is a higher degree of variance in the structural composition, as quantified by UTC, at the proximal patellar tendon attachment in the pre and peri-PHV participants; 3) the tendon attachment has a similar appearance in the post PHV and mature group; 4) the greyscale classification system corresponded to the latter stages of maturity offset (post-PHV and mature) with the earlier stages appearing more variable.
This study showed that the appearance of the tendon attachment differed before and after peak height velocity on greyscale imaging. The variable and more hypoechoic appearance in pre- and peri-PHV participants became more normechoic and continuous across the patella in the sagittal and transverse plane in post-PHV and mature groups. While we are limited by these cross-sectional data, the progression towards normal echogenic imaging on US appeared to occur in an anterior to posterior direction with maturity. The posterior aspect may be the last region to mature, which may make it vulnerable to mechanical loading in the pre and peri-PHV years. Interestingly, hypoechoic areas on ultrasound imaging are frequently observed on the posterior aspect at the patellar insertion in adults with patellar tendinopathy[23-25]. One possible explanation is that the hypoechogenicity is a normal part of tendon attachment development that will disappear with normal skeletal maturation, or if exposed to excessive tensile and/or compressive loads before it has formed a mature attachment, it could develop a pathological attachment.
This progression of normal development has been observed at the tibial end of the patellar tendon; a hypoechoic area on ultrasound is common around peak height velocity and less common post-PHV, demonstrating normal tendon tissue maturation[11]. Similar to the findings at the distal patellar tendon attachment[11], the implication for the imaging finding may be that not all hypoechoic areas are pathological in pre and peri PHV athletes but rather these areas may be a sign of skeletal immaturity that will "normalise" following PHV. During adolescence the patellar tendon's tibial attachment is vulnerable to the development of pain which, at this site, is termed Osgood-Schlatter disease (OSD). Using grey-scale ultrasound imaging, Ducher et al. (2010) demonstrated that the tibial end of the patellar tendon matures during puberty. The tibial attachment is a cartilage attachment ~1.8 years before PHV, enthesial fibrocartilage immediately after PHV, where a mature attachment is seen ~2 years after PHV[10, 11]. Interestingly, the development of pain appears to be related to certain stages of the maturation process of the tibial insertion, suggesting that aberrant loading during maturation can have a negative effect on the tendon attachment[26].
The presence of tendon pathology on imaging represents some risk factor for developing patellar tendon

Table I. Mean anterior-posterior patellar tendon thickness (in centimeters) by maturity offset category.

Maturity Offset Categor	Inferior Pole	1 cm Distal *	2 cm Distal **
Pre	.44	.44	.43
Peri	.45	.44	.44
Post	.48	.47	.45
Mature	.47	.54	.51

Significantly increasing thickness with increasing maturity offset category:
*P=0.001 at 1 cm
**P=0.007 at 2 cm
(P=0.132 at the inferior pole)

Table II. Greyscale score group incidence by maturity offset category.

Maturity Offset category (n)	**Greyscale category 1**	**Greyscale category 2**	**Greyscale category 3**
Pre (21)	3 (14%)	11 (52%)	7 (33%)
Peri (27)	0 (0%)	17 (63%)	10 (37%)
Post (9)	1 (11%)	2 (22%)	6 (67%)
Mature (8)	0 (0%)	1 (12.5%)	7 (87.5%)

Table III. One-way ANOVA of mean echo-type pattern (with standard deviation included, SD) at various distances from inferior pole of patella by maturity offset groups. Echo-type I being the most aligned, echo-type IV the least aligned.

Distance from inferior pole (cm):	Echo-type	Mean quantity of echotype Pre-PHV (SD)	Mean quantity of echotype Per-PHV (SD)	Mean quantity of echotype Post-PHV (SD)	Mean quantity of echotype Mature (SD)	P-Value (CI=95%)
0-1	I	.56 (.12)	.54 (.12)	.63 (.09)	.59 (.05)	.210
0-1	II	.42 (.11)	.45 (.11)	.36 (.08)	.40 (.06)	.187
0-1	III	.01 (.01)	.01 (.01)	.01 (.01)	.01 (.01)	.752
0-1	IV	.00 (.01)	.00 (.00)	.00 (.00)	.00 (.00)	.504
1-2	I	.68 (.13)	.68 (.09)	.77 (.07)	.70 (.04)	.130
1-2	II	.32 (.13)	.31 (.09)	.23 (.07)	.29 (.05)	.135
1-2	III	.00 (.01)	.00 (.00)	.00 (.00)	.01 (.00)	.214
1-2	IV	.00 (.01)	.00 (.00)	.00 (.00)	.00 (.00)	.574
0-2	I	.62 (.11)	.61 (.10)	.70 (.07)	.65 (.04)	.127
0-2	II	.37 (.12)	.38 (.10)	.30 (.07)	.34 (.05)	.119
0-2	III	.01 (.01)	.01 (.01)	.00 (.00)	.01 (.00)	.714
0-2	IV	.00 (.01)	.00 (.00)	.00 (.00)	.00 (.00)	.495

pain[1, 27]. However, there are a multitude of factors that also contribute to onset of symptoms, where mechanical overload has been shown to be an important risk factor[28-34]. The patellar tendon is an energy-storage tendon that is loaded by jumping; hence why symptoms are called "jumper's knee." High volumes of jumping and energy-storage may induce pathology or trigger symptoms in a tendon that is pathological.

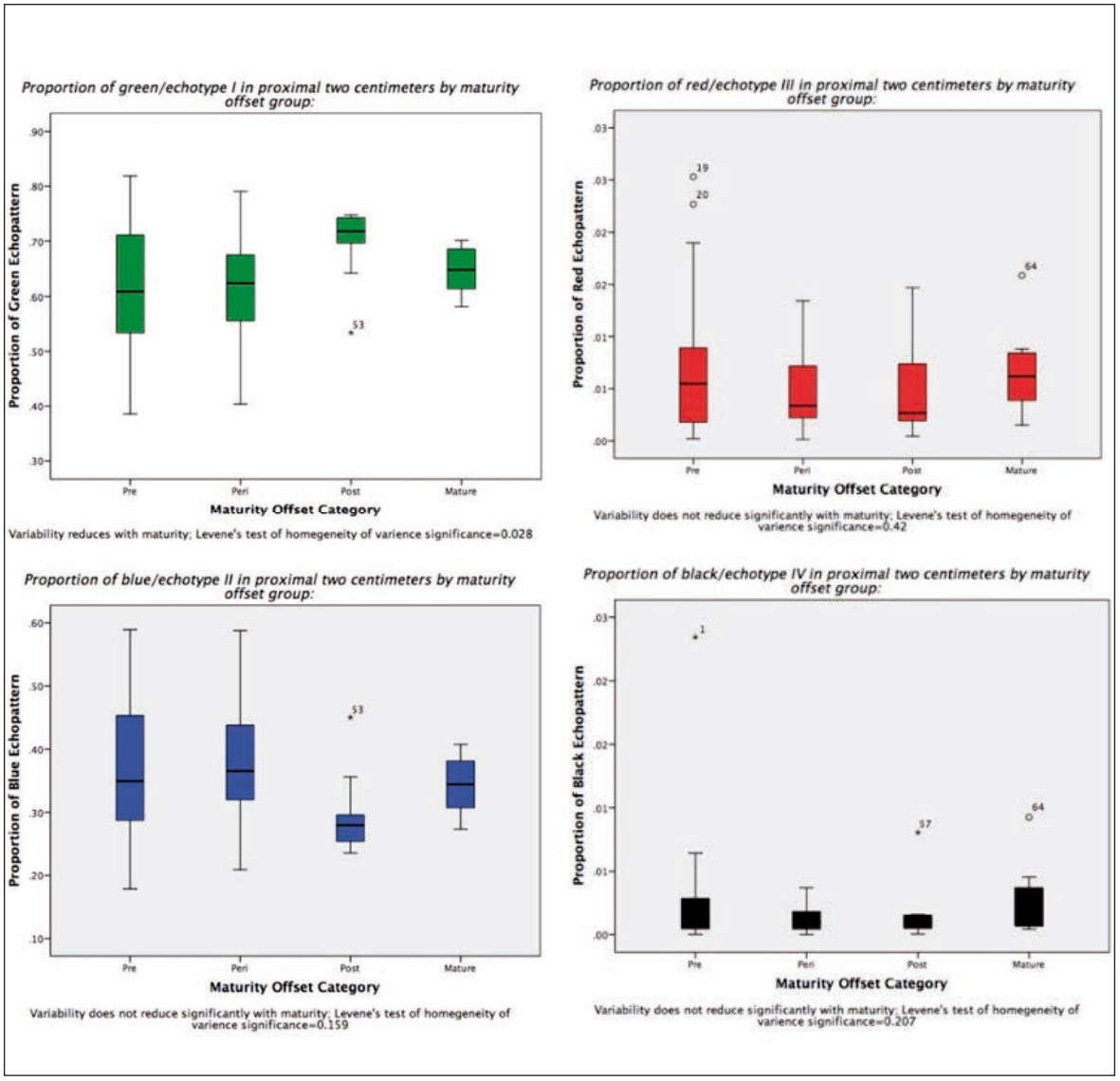

Figure 4. Variance of echopatterns.

Interestingly, dancers complete approximately 200 jumps in each ballet class that may induce pathological changes[35]. While energy-storage is the primary load placed on the patellar tendon, the patellar insertion may also have compressive loads that may be implicated in the development of pathology[36]. Hamilton and Purdam (2004) described hypoechoic areas on the posterior aspect of the attachment as an adaptive response to compressive loads and a potential variation in how the tendon inserts into the patella[23].

This study also reported that the tendon is thicker at 1 and 2 cm distal to the inferior pole of the patella in mature participants. This is a similar result to the finding of Kubo et al. (2014) who observed in a cross-sectional study that the cross-sectional area and length of the patellar tendon increased with larger body size during growth[12]. It is likely that tendon thickness is an adaptation to increasing body mass and muscle strength during puberty.

The variability in the echopattern decreased through maturity at the patellar insertion, particularly echotypes I and II. Based on our current understanding of tendon maturation, it is possible that hyaline or fibrocartilage with type II collagen and larger proteoglycans are present in younger people, which then transitions to mature tendon tissue[37].

Clinical implications

Based on these preliminary findings, we see that while there is a high degree of variation in the appearance of the proximal patellar tendon attachment in pre and peri PHV groups, the post PHV tendons appear mature. This supports our hypothesis that there is a transition towards mature tendon attachment during PHV, which is important because once clinicians better understand normal development;

they can begin to understand abnormal or pathological development, which can lead to pain and dysfunction. This study also provides a method to describe the variations in tendon appearance using the greyscale score with the option to describe all three planes within the tendon. Prospective studies are needed to validate whether the proximal patellar tendon attachment develops during puberty as is proposed and suggested by the current study.

Limitations of this study include only one blind assessor and one blind reviewer evaluating the UTC scans where other studies have used three blind assessors, though reliability of the UTC has been established in previous studies[17]. The greyscale score needs to be validated in a prospective study. Also, there is an unequal distribution of participants between the three peak height velocity groups and the mature groups.

Conclusions

Based on this cross-sectional study, we found that leading up to peak height velocity the tendon has a variable appearance compared to post peak height velocity. While tendon development is not solely dependent on peak height velocity and other factors may influence maturation, it does appear that pre and peri PHV are important stages for the transition of the proximal patellar tendon attachment to mature tendon. Prospective research is needed to improve our understanding of how the proximal patellar tendon attachment develops throughout skeletal maturity, and whether the stage of tendon development impacts the risk of developing tendon pathology as has been shown at the distal attachment[10, 26].

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Currently under review at Scandinavian Journal of Medicine & Science in Sports

Title: Quantifying proximal patellar tendon changes during adolescence in elite ballet dancers, a 2-year study.
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Abstract:
Purpose: Patellar tendon pathology appears to develop in young athletes. It is not known how this tendon develops through adolescence. This longitudinal study investigated proximal patellar tendon development during the adolescent growth spurt in young ballet dancers, and identified if puberty (estimated by maturity offset) had an effect on tendon development. **Methods**: 52 dancers had ultrasound images of their tendons every 6 months for 2 years. Changes in tendon size (anterior-posterior diameter) on greyscale images and echogenicity, as quantified using ultrasound tissue characterization, were recorded each time. Maturity offset was calculated and used to estimate peak height velocity (adolescent growth spurt). **Results**: Maturity offset did not affect effect tendon composition before peak height velocity, however after participants passed peak height velocity, maturity offset increased the composition of stable echopattern ($p<0.05$); a 4% differential increase in type I echopattern, indicative of normal tendon structure, and a decrease in type III echopattern (more disorganized echopattern) by 0.7% per year. Anterior-posterior thickness increased by 0.2mm/year ($p<0.05$) measured 2cm below the patella. **Conclusions**: Following peak height velocity, the proximal patellar tendon attachment increased in thickness and demonstrated a more stable echopattern representative of aligned fibrillar structure. Future research is required to further understand this normal maturation and the factors that support this process, with the aim of reducing the development of patellar tendon pathology in the adolescent jumping athlete.

Key Words: Tendon development, peak height velocity, Ultrasound Tissue Characterization

INTRODUCTION

Tendinopathy refers to the clinical diagnosis of tendon pain and dysfunction, whereas tendon pathology is structural change within the tendon tissue, usually identified on ultrasound or MRI (1). Lower limb tendinopathy and tendon pathology are seen in adolescents, with the incidence of symptoms increasing up to 18 years of age (2). Pathology at the proximal attachment of the patellar tendon has been shown to increase risk of developing pain (patellar tendinopathy (PT)) (3-5), commonly known as jumper's knee. PT is frequently observed in athletes of sports that involve high volumes of jumping, such as basketball, volleyball and dance. The prevalence of tendon pathology in adolescent basketball players (aged 14-18 years old) is similar to the adult population (26% in adolescents compared to ~30% in adults (4)), suggesting that the normal tendon maturation during adolescence is disrupted leading to the development of tendon pathology, which may remain into adulthood.

Current understanding of how the proximal patellar tendon matures is based on sparse literature about general tendon development. Tendons elongate and thicken at a rate proportional to the growth of their associated muscles and bones (6-8). The adolescent growth spurt during puberty is a period of rapid growth that may be a critical time for tendon maturation. Puberty can be quantified by measuring the peak growth of the adolescent growth spurt (peak height velocity (PHV)) that is typically achieved around 12 years old for girls and 14 for boys (9) and maturity offset is the distance, in years, from peak height velocity. Puberty is a crucial time to assess the proximal patellar tendon attachment as tendon tissue must also mature by the end of puberty as tendon collagen has been shown to be relatively inert after age 17 (10). Understanding the normal maturative process of the proximal patellar tendon is critical, as disrupting this process may lead to the development of pathology and symptoms, as has been shown at the distal attachment (11, 12).

Little research has looked at the maturation of the patellar tendon and the formation of the proximal and distal patellar tendon attachments. Ducher et al looked at the distal attachment of the patellar tendon to the tibia during adolescence and showed mature attachments formed on average 2 years after PHV in tennis players (11, 12). Disruption to normal distal tendon development at this critical stage of maturation has been associated with pain (Osgood-Schlatter disease) (13). To investigate whether disruption of the normal development of the proximal tendon leads to pain, a clear understanding of the formation of a mature proximal patellar tendon attachment during adolescence is critical.

Tendon maturation can be measured by quantifying tendon thickness using conventional greyscale ultrasound. Patellar tendon thickness has been positively correlated with age in 11-15 year old volleyball players (14). However, the effect of puberty is unclear as no significant difference in patellar tendon thickness was found in pre-pubertal versus post-pubertal 13-year-old athletes (15). In a cross-sectional study, the proximal tendon attachment in participants who were less than 1 year post peak height velocity had variable appearances and greyscale scores, while those who were more than 1 year post PHV had tendons appearances and greyscale scores similar to mature participants between 21-40 years old (16). These findings suggesting that the period around peak height is critical in the formation of an organized and mature tendon attachment. A limitation of some of these previous studies was that they relied on

subjective measures of tendon structure, where new imaging modalities allowing for quantification of structure may provide further insight and objectivity.

Ultrasound tissue characterization (UTC) is an ultrasound based imaging modality, which has been used to investigate the clinical presentation of tendinopathy, but has also provided insight into subtle changes in response that have previously not been detected by conventional imaging (17, 18). Based on the consistency of the greyscale image along the length of the tendon, four echotypes are generated that can be quantified as a percentage. These echotypes have been validated and shown to discriminate between different pathological states (19, 20), as well as exhibiting excellent interobserver reliability (20) and intraobserver reliability (21). UTC may be a useful tool in investigating the maturation of the tendon, as it does not rely on subjective interpretation.

The aim of this longitudinal study was to observe the proximal patellar tendon development during the adolescent growth spurt in healthy ballet dancers and to determine the effect of maturity offset on the tendon over time. Ballet dancers were selected because they have a high jumping volume in their sport, which loads the patellar tendon, and they have a low attrition rate, which allowed for follow up over a 2-year period. The hypothesis was that there would be an interaction between maturity offset and changes in tendon thickness and composition.

METHODS

Ballet students from the Australian Ballet School and Victorian College of the Arts Secondary School (11-18 years of age at the start) participated in this study. Maturity at baseline varied from pre-pubertal to post pubertal, post-pubertal participants were included to examine if change in tendon structure occurred after puberty. All participants were regularly involved in 4-8 hours of daily dance classes. A typical 1.5 hour ballet class can include 200 jumps (22). Data were collected every 6 months for 2 years, five time points in total. As this study aims to investigate normal tendon maturation, dancers exhibiting tendon pathology (indicated by the presence of a hypoechoic area at more than one data collection point) were excluded. Knee pain was not considered as this study was primarily focused on the structure of the tendon during puberty. Monash University ethics committee approved this study and all participants, or if aged under 16 years the participants' parents, gave informed consent.

Anthropometric measurements:

Height (cm) and sitting height (cm) were measured with a stadiometer, and weight using electronic scales (kg), were collected at each data point. Sitting height was measured with participants sitting tall on a table, thighs fully supported on the table and feet supported with knees flexed to 90°. The measurement of sitting height was taken from the ischial tuberosity to the top of the participant's head. Leg length was calculated by subtracting sitting height from standing height. The interaction between leg length and trunk growth rate changes during the adolescent growth spurt and can be estimated with peak height velocity (PHV) (9). Maturity offset is calculated based on the equation by Mirwald, et al. and is the number of years a person is away from his PHV (9). Participants were categorized as either pre or post peak height velocity if their maturity offset was negative or positive at baseline respectively.

Ultrasound Tissue Characterization:

Ultrasound tissue characterization (UTC) scans were performed on each participant's left knee, which was flexed to 90° while the participant was in a supine position. Scans were done by one of two investigators (AR or SD). A linear-array ultrasound transducer (SmartProbe 10L5, Terason 2000+; Teratech) mounted in a tracking device with a built-in acoustic-coupling stand-off pad (UTC Tracker, UTC Imaging) was placed on the patellar tendon. Once a clear image of the tendon and inferior pole was apparent, the transducer moved automatically distally over the length of the tendon capturing 600 transverse greyscale image every 0.2mm.

UTC software utilized these 600 contiguous transverse ultrasound images to render a three-dimensional greyscale image (19). The dedicated software analyses the stability of the echopattern across multiple transverse images categorizing the tendon into four echo-types (UTC2010, UTC Imaging): echo-type I (green) is the most stable, echo-type II (blue) is stable but slightly less organized than type I, echo-type III (red) is slightly disorganized tissue and echo-type IV (black) being the most disorganized (19) (19, 20, 23). Histopathological specimens from the horse have been correlated with UTC echo-types to show it is a valid modality to estimate pathology (19). UTC also demonstrates excellent inter and intra observer reliability with ICC of 0.95 for determining echo-types I and II (20).

The region of interest (ROI) for the patellar tendon was selected from the disappearance of the inferior pole of the patella, extending 2 cm distally. Manually selected contours were placed along the length of the tendon in intervals no more than 4 mm. The UTC software automatically interpolated contiguous ROIs creating a tendon volume where the proportions of each echo-type were calculated (0-1cm from the inferior pole and 1-2cm from the inferior pole). Quantification of the UTC echo-type was performed by the same investigator (AR) blinded to participant identity and age, with the window size set at 25 (slice-thickness 4.8mm). Contours were reviewed by another trained researcher (SD) to ensure consistency.

Greyscale Score:

A classification system developed to categorize the appearance of normal tendon attachments on greyscale imaging were based on greyscale imaging features at different maturity levels in the sagittal, transverse and coronal planes (16). The tendon was scored between 0-2 based on appearance in each of the three planes, where tendon fibers were classified as either clearly not attached to the patellar (score 0), through to clearly attached (score 2). The total greyscale score is between 0-6, where 0 represents an immature tendon appearance and 6 represents a fully mature tendon appearance.

Anterior-Posterior Thickness:

Anterior-posterior thickness (mm) was measured in the axial plane using the greyscale ultrasound images on the UTC. Measurements were taken at the inferior pole of the patella, 1 and 2 cm distal.

Statistical analysis:

Analyses were done using the plm package in the R statistical package. Participants were excluded if they missed more than two data points and all missing time points were removed from analyses. Analysis was completed using a random effects multivariate longitudinal data regression model with an interaction term to test if there was a differential effect of maturity offset on the outcomes of interest: (echopattern by type (I-IV) at 1 and 2 cms distal to the proximal attachment, anterior-posterior thickness

at the inferior pole, 1 and 2 cms distal, and greyscale score) after participants reached peak height velocity. The random effects longitudinal model allows analysis of between and with-in individual variation of longitudinal data (24). For this analysis, maturity offset was the independent variable and each of the previously mentioned outcomes were the dependent variables. The purpose of the interaction term was to determine if maturity offset had a greater marginal effect on the outcomes after the participants had passed peak height velocity. The regression model is as specified in the following equation:

$$y_{it} = \alpha + \beta_1 MaturityOff + \beta_2 Maturity\ OffxPeak\ Height\ + \epsilon_{it}$$

y_{it} represents the outcome measures of interest, $MaturityOff$ is the maturity offset measure (measured in years), $PeakHeight$ is an indicator variable equal to one if the participant is past peak height velocity at the start of the study (16 participants) and zero otherwise (36 participants), and, ϵ_{it} represents the idiosyncratic error term. In the equation β_1 represents the primary effect of maturity offset on the outcome of interest and β_2 represents the differential effect of maturity offset if a participant is past peak height velocity. Therefore, the total effect of maturity offset on the outcome of interests for participants past peak height velocity is $\beta_1 + \beta_2$.

Additionally, to measure the change in Greyscale score over time, Greyscale Score was regressed on time periods relative to the baseline score.

RESULTS

61 participants were recruited for this study, 9 were excluded because of missing data or hypoechoic areas. Of the remaining 52 participated, 32 were women and 20 men, with an age range at baseline 11-18 years. Dancers participated in 10-27 hours of dance class and rehearsal per week, increasing with age. Of the female participants, 24 were pre-PHV at baseline and 8 were post-PHV. Of the 20 male participants, 12 were pre-PHV at baseline.

Ultrasound Tissue Characterization:

Once participants were post-PHV, maturity offset had a positive relationship with the percentage of echo-type I, indicating increases in normal aligned tendon bundles after PHV. There was also a significant relationship between echo-type III, representing disorganized fibrillar tissue, and maturity offset on participants post PHV (Figures I and II). No significant relationship was observed between any echo-type and maturity offset beginning prior to PHV. These findings were similar for both the first and second centimeters of the tendon. In both the first and second centimeter, a marginal increase of 4.2% and 4.0% of echo-type I was observed after PHV (Tables I and II).

Greyscale score:

The greyscale score increased as the group transitioned through adolescence but maturity offset showed no significant effects on the greyscale score (Table III).

Anterior-Posterior Thickness

Tendon thickness 2cm distal to the inferior pole was associated with maturity offset, regardless of whether participants were pre- or post-PHV at the start of the study. The tendon thickened at a rate of 2 mm/year in second cm (Table IV). The participants also demonstrated an increased thickness throughout the tendon when they reached peak height velocity (Table IV).

DISCUSSION

This study demonstrates a transition towards more stable and mature tendon tissue post-PHV in adolescent jumping athletes with normal patellar tendons. There was a greater marginal effect of maturity offset on the UTC echopattern after peak height velocity, which showed increases in echo-type I in the proximal 2 centimeters of the tendon. These UTC changes suggest improvements in tendon structure. These findings are consistent with the hypothesis that maturative structural changes are related to peak height velocity.

Anterior-posterior thickness increased in the patellar tendon during adolescence in the second centimeter of the tendon with maturity offset. Considering that PHV measures the accelerated growth in long bones of the lower limb, it is consistent that after this growth spurt, the patellar tendon would thicken. The findings in this longitudinal study are consistent with a previous cross sectional study (16) however it is inconsistent with another study, which found no change in patellar tendon thickness in asymptomatic tendons over adolescence (5) though participants were already 15-16 years old at the start of that study. Increasing tendon thickness may be an adaptive mechanism to cope with the sudden increase in femur length and muscle mass that increases tensile loading on the tendon.

A previous cross sectional study found greyscale scores varied in the pre and peri peak height velocity participants while post PHV participants had subjective features of a mature tendon attachment (16). Interestingly, this longitudinal study showed that there was no relationship between greyscale score and maturity offset.

Peak height velocity and the adolescent growth period is a critical time for changes within tendon structure and thickness. This study supports the concept of tendon maturation occurring after peak height velocity, before tendon collagen turnover is limited around age 17 (10). Because tendons that appear normal by age 17 are at a low risk for developing pathology or symptoms later in life (25), this maturation process after PHV is an important developmental stage for ballet dancers, and potentially other jumping athletes. There is potential to use these data for managing load on adolescent tendons, where key periods of tendon development are identified through measures of puberty, and jumping loads managed to ensure normal tendon development.

Ensuring healthy and normal tendon development in adolescent athletes requires identification of when these changes occur. This study supports the hypothesis that the adolescent growth spurt is the critical transition phase in proximal patellar tendon maturation. Our findings at the proximal patellar tendon attachment are consistent with the findings at the distal patellar tendon attachment, which showed a transition to a mature attachment 2 years after peak height velocity (11, 12). In order to promote developing optimal tendon structure, future research must investigate factors that contribute to or inhibit normal tendon development.

LIMITATIONS

Some participants in this study were not pre peak height velocity at the start of the study. Other measures of adolescent growth may be more accurate than PHV, however methods such as Tanner staging and wrist X-ray are ethically difficult. Accuracy of the Mirwald equation in lower body weight female athletes who may be late to maturity is

not known. The minimal detectable change in patellar tendons for a UTC scan was identified as 1.7% of aligned and disorganized structure (26). While the UTC has not been validated in humans, it has been validated in horse tendons, which are similar to human tendons (19, 27, 28). Additionally, the greyscale score has not yet been validated.

PERSPECTIVES

This study adds to the understanding of how and when the proximal patellar tendon matures during adolescence in the athletic population. The period after the adolescent growth spurt is a critical time for the proximal patellar tendon to develop, with changes observed on UTC indicative of improvements in tendon structure. These findings are important for young athletes, coaches and medical professionals to promote healthy tendon maturation. More research is needed to identify factors that support this normal development or those that can lead to the development of pathology in young jumping athletes.

Acknowledgements: The authors thank Janet Karin of the Australian Ballet School and Margaret Storey of the Victorian College of the Arts Secondary School for their help in coordinating and facilitating data collection. The authors also thank Daniel Brent, PhD for consulting with statistical analysis. The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation, and do not constitute endorsement by ACSM.

Disclosures: There are no conflicts of interest of financial disclosures.

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Tables and Figures:

Figure I: Effect of maturity offset on change in echopattern in the proximal first centimeter:

Key: Black (Type IV), Blue (Type II), Green (Type I), and Red (Type III)

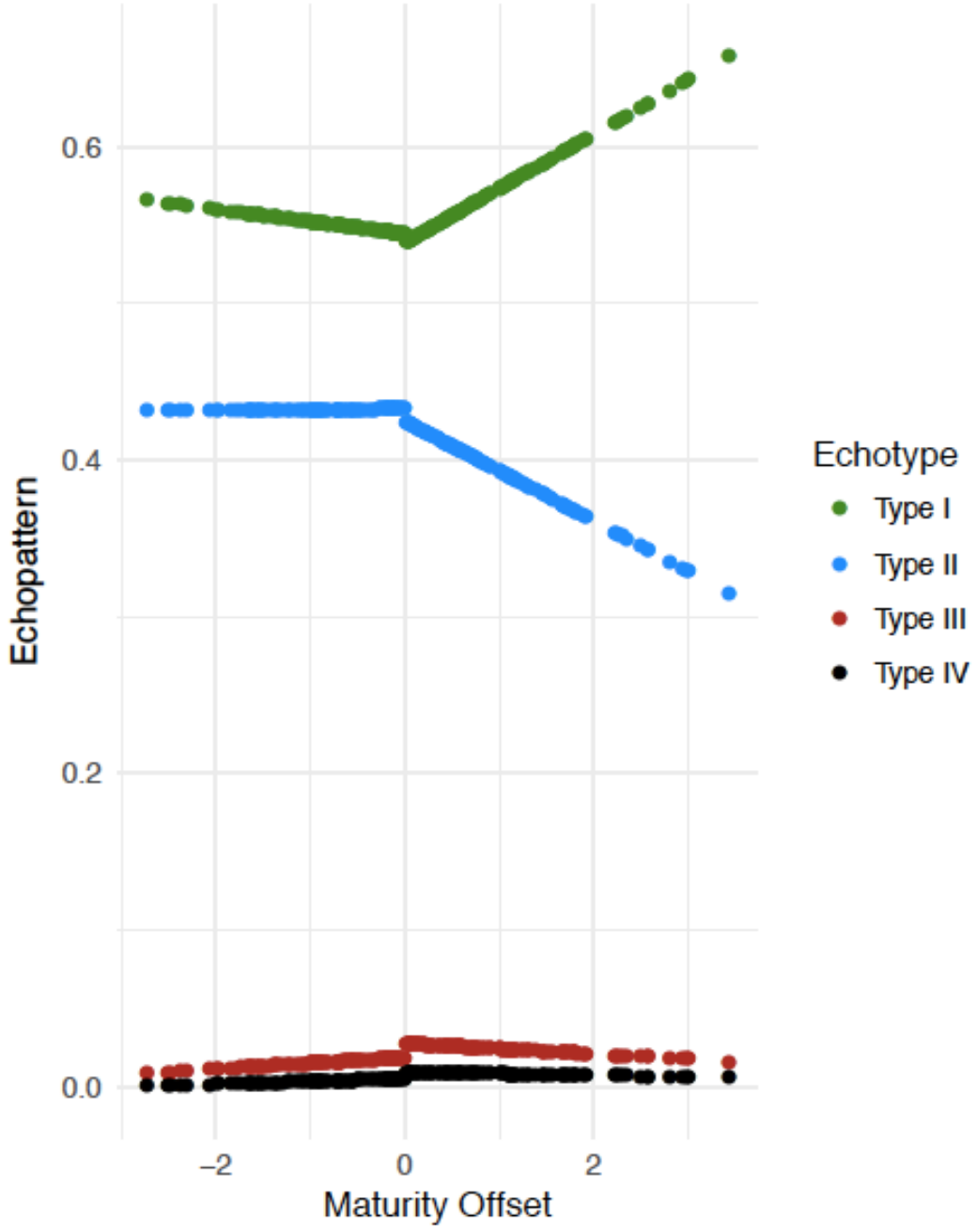

Figure II: Effect of maturity offset on change in echopattern in the proximal second centimeter:

Key: Black (Type IV), Blue (Type II), Green (Type I), and Red (Type III)

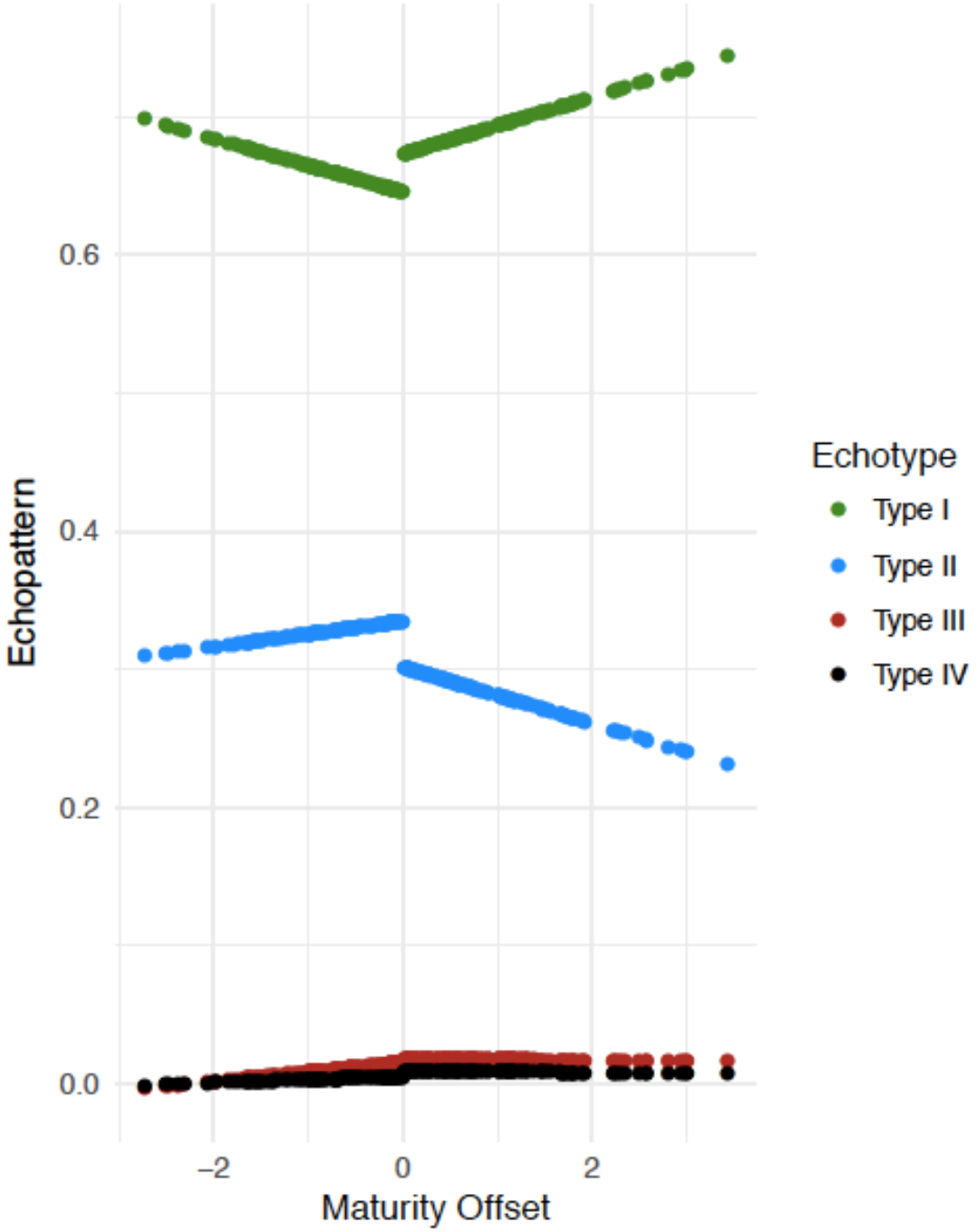

Table I: Echopattern in First Centimeter:

	Type I (Green)	Type II (Blue)	Type III (Red)	Type IV (Black)
Maturity Offset	-0.008	0.001	0.004	0.002
	(0.015)	(0.015)	(0.003)	(0.002)
Peak Height	-0.006	-0.008	0.009	0.004
	(0.020)	(0.021)	(0.005)	(0.002)
Interaction Effect	0.042**	-0.032	-0.007	-0.003
	(0.020)	(0.020)	(0.004)	(0.002)
N	222	222	222	222
R2	0.096	0.078	0.049	0.049
Adjusted R2	0.084	0.065	0.036	0.035
F Statistic (df = 3; 218)	7.749***	6.128***	3.744**	3.709**

Notes:
***Significant at the 1 percent level.
**Significant at the 5 percent level.

Table II: Echopattern in Second Centimeter:

	Type I (Green)	Type II (Blue)	Type III (Red)	Type IV (Black)
Maturity Offset	-0.019	0.009	0.007***	0.002
	(0.013)	(0.013)	(0.003)	(0.001)
Peak Height	0.027	-0.033	0.003	0.004
	(0.018)	(0.017)	(0.003)	(0.002)
Interaction Effect	0.040**	-0.030	-0.007**	-0.003
	(0.017)	(0.017)	(0.003)	(0.002)
N	222	222	222	222
R2	0.128	0.109	0.077	0.068
Adjusted R2	0.116	0.096	0.064	0.055
F Statistic (df = 3; 218)	10.473***	8.825***	6.031***	5.284***

Notes:
***Significant at the 1 percent level.
**Significant at the 5 percent level.

Table III: The Effect of Time on Change in Greyscale Score:

	Greyscale_Score
Time 2	0.595**
	(0.238)
Time 3	0.424**
	(0.213)
Time 4	1.172***
	(0.222)
Time 5	-2.053***
	(0.231)
N	216
R2	0.559
Adjusted R2	0.408
F Statistic	50.760*** (df = 4; 160)

Notes: ***Significant at the 1 percent level.
**Significant at the 5 percent level.

Table IV: Anterior-Posterior Diameter:

	AP Diameter (in mm)		
	Inferior Pole	1cm Distal	2cm Distal
Maturity Offset	0.010	0.183	0.199**
	(0.091)	(0.103)	(0.098)
Peak Height	0.280**	0.364***	0.294**
	(0.126)	(0.138)	(0.129)
Interaction Effect	0.009	-0.233	-0.076
	(0.118)	(0.135)	(0.130)
N	220	220	220
R2	0.133	0.174	0.209
Adjusted R2	0.121	0.162	0.198
F Statistic (df = 3; 216)	10.679***	15.076***	19.034***

Notes: ***Significant at the 1 percent level.
**Significant at the 5 percent level.

Currently revise and resubmit at Scandinavian Journal of Medicine & Science in Sports

Title: Proximal patellar tendon pathology can develop during adolescence in young ballet dancers- a 2 year longitudinal study.

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Abstract:

Purpose: Pathology in the proximal patellar tendon is a key risk factor for developing tendon pain and dysfunction, this is known as patellar tendinopathy, or jumper's knee and is seen in adult jumping athletes. When pathology develops in the proximal patellar tendon is not known, though it is reported to exist in adolescent athletes. The aim of this study was to follow young jumping athletes (ballet dancers) through adolescence to identify if pathology develops and its relation to the adolescent growth spurt.
Methods: 57 elite ballet students between ages 11-18 were monitored for 2 years. Data was collected every 6 months, including: an ultrasound scan on their left tendons using Ultrasound Tissue Characterization (UTC) to quantify intra-tendinous changes, anthropometric data to calculate peak height velocity (adolescent growth spurt), participant reports of any injuries or dance modifications and a VISA-P and single leg decline squat for patellar tendon pain.
Results: 9% of adolescent dancers developed pathology during this study. There were no differences between those who did not develop pathology and those who did in peak height velocity or dance participation/volume either at the start or throughout the study. It was not associated with a specific time in puberty. Only two out of five participants who developed pathology reported pain associated with their tendon.
Conclusions: Pathology on the proximal patellar tendon can develop during adolescence. It was not correlated with the onset of pain and dysfunction.

Key words: Jumper's knee, dance medicine, skeletal development

Introduction

Patellar tendinopathy (PT), known as "jumper's knee", is a condition of pain and dysfunction at the proximal patellar tendon associated with overuse in jumping and cutting sports. The prevalence of jumper's knee has been reported to range from 11.8% to 36% in jumping sports such as basketball and volleyball (1, 2). A risk factor for developing patellar tendinopathy is the presence of pathology at the proximal patellar tendon on ultrasound or magnetic resonance imaging (3-6).

Tendon pathology observed on imaging has been shown to increase the risk of developing painful tendinopathy 4-fold in patellar tendons (7). However, a large portion of those with pathology remains asymptomatic, suggesting that pathology may develop prior to the presence of clinical symptoms (6, 7). In the elite adult ballet population, 12% of dancers (n=19) showed moderate to severe hypoechoic areas on their patellar tendons, yet only 3 out of the 19 dancers with hypoechoic areas developed jumper's knee within the following 2 years (3).

Patellar tendon pathology may develop during adolescence in jumping athletes (8, 9). By 16 years of age, 10% of asymptomatic elite volleyball players had pathology within their proximal patellar tendons (6), another study reported that 29% of junior volleyball players had tendon pathology on ultrasound (10). Little is known about how or when pathology develops in the tendon, though adolescence appears to be a critical time in patellar tendon development (11).

Adolescent ballet dancers' proximal patellar tendons appear mature around 1-2 years after their adolescent growth spurt (11). Similar timing was seen at the distal patellar tendon attachment in adolescent tennis players (12, 13). As tendon collagen becomes relatively inert around 17 years of age (14), pathology likely develops during adolescence. If normal proximal patellar tendon maturation occurs during adolescence and the development of pathology is unlikely after adolescence, then perhaps abnormal or pathological tendon development occurs during this time as well. To date, few studies have looked at the development of normal patellar tendons during adolescence, and no longitudinal studies have identified when or how patellar tendon pathology develops. It is critical to understand how and when pathology initially develops as it is a key risk factor for the onset of symptoms.

Pathology on imaging is the primary intrinsic risk factor for developing pain (3, 6, 15). The primary extrinsic risk factor for pathology and pain is overload/increased training volume (16-18). Identifying when pathology develops and its associated risk factors may aid in designing prevention strategies in jumping athletes to reduce the risk of pathology, pain, dysfunction, and possible withdrawal from sports participation (19).

The aim of this study was to prospectively follow adolescent ballet dancers over a 2-year period to identify if patellar tendon pathology develops in this time. The secondary aim was to investigate if skeletal maturity, load volume and pain were associated with the development of tendon pathology. The primary hypothesis was that a portion of participants would develop pathology during adolescents. The second hypothesis was that there would be a difference in skeletal maturity, load volume and pain between those who do and those who do not develop pathology.

Methods

Participants:

Ballet students from the Australian Ballet School and Victorian College of the Arts Secondary School (11-18 years of age at baseline) participated in this study. All participants were regularly involved in 4-8 hours of daily dance classes. A typical 1.5 hour ballet class can include 200 jumps (20). Data were collected every 6 months for 2 years, five time points in total. Dancers were excluded from the study if they missed two or more time points of data collection. Past medical history and medication list were collected at baseline. Monash University ethics committee approved this study and all participants, or participants' parents if aged less than 16 years, gave informed consent.

Ultrasound Tissue Characterization:

Ultrasound tissue characterization (UTC) scans were performed on each participant's left knee while the participant was in a supine position with knee flexed to 90°. Scans were performed by one of two investigators (AR or SD). A linear-array ultrasound transducer (SmartProbe 10L5, Terason 2000+; Teratech) mounted in a tracking device with a built-in acoustic-coupling stand-off pad (UTC Tracker, UTC Imaging) was placed along the axis of the patellar tendon. Once a clear image of the tendon and inferior pole was visualized, the transducer moved distally and automatically over the length of the tendon, capturing 600 transverse greyscale images every 0.2mm.

Dedicated UTC software renders a three-dimensional greyscale image from the 600 contiguous transverse ultrasound images (21). The software analyses the stability of the echopattern across multiple transverse images, and categorizes the tendon into four echo-types (UTC2010, UTC Imaging): echo-type I (green) represents the most stable, echo-type II (blue) represents stable but slightly less organized than type I, echo-type III (red) represents slightly disorganized tissue and echo-type IV (black) represents the most disorganized (21) (21-23). Histopathological specimens from the horse have been correlated with UTC echo-types demonstrating it is a valid modality to estimate pathology (21). Excellent inter- and intra-observer reliability of UTC has also been demonstrated with ICC of 0.95 for determining echo-types I and II (23).

The region of interest (ROI) for the patellar tendon was selected from the disappearance of the inferior pole of the patella, extending 1 cm distally. Contours were selected manually and placed along the length of the tendon in intervals no more than 4 mm. The UTC software automatically interpolated contiguous ROIs creating a tendon volume where the proportions of each echo-type were calculated. The window size was set at 25 (slice-thickness 4.8mm). The same investigator (AR) performed the quantification of the UTC echo-type and was blinded to participant identity and age. A second trained researcher (SD) reviewed the contours to ensure consistency. For the analysis, type III (red) and type IV (black) echopattern were combined and considered "disorganized tissue."

Determining Pathology/Hypoechoic Area:

At each time point a rater (AR), blinded to participant's details, determined if there was a focal hypoechoic lesion on greyscale ultrasound imaging. Ultrasound images were classified as either having a tendon hypoechoic area or not at the

proximal attachment. A second blinded rater (SD) confirmed the determination. Dancers were categorized as having an abnormal tendon if they had a hypoechoic area on greyscale ultrasound for two or more time points (pathology group), if not, they were considered to have a normal tendon (normal group).

Greyscale Score:

A greyscale score was developed by Rudavsky et al, to describe the appearance of the tendon on greyscale ultrasound, which has been shown to be associated with peak height velocity (11). This score described features of the proximal patellar tendon in the sagittal, frontal, and transverse planes. The tendon was scored between 0-2 based on appearance in each of the three planes, where tendon fibers were classified as either clearly not attached to the patellar (score 0), through to clearly attached (score 2). The total greyscale score is between 0-6, where 0 represents an immature tendon appearance and 6 represents a fully mature tendon appearance (11). The greyscale score was only calculated for normal tendons.

Anthropometric measurements:

Height (cm), weight (kg) and sitting height (cm) were collected at each data point. A stadiometer was used to collect height and sitting height (cm), and an electric scale was used for weight (kg). For sitting height, participants were sitting tall on a table, thighs fully supported on the table and feet supported with hips and knees flexed to 90°. The measurement of sitting height was taken from the ischial tuberosity to the top of the participant's head. Leg length was calculated by subtracting sitting height from standing height. Using these measures, maturity offset was calculated to estimate peak height velocity (PHV) based on the equation by Mirwald et al. (24). A negative maturity offset value is interpreted as how many years prior to peak height velocity (adolescent growth spurt) the participant was and a positive maturity offset signified years past peak height velocity. Maturity offset was calculated at each time point.

Pain:

Participants completed the Victorian Institute of Sport Assessment for the Patellar Tendon (VISA-P) questionnaire and performed the visual analogue scale (VAS) for pain below the patella while performing a single leg decline squat (SLDS) at all time points. The single leg decline squat involved performing a single leg squat to 60° on a 25° decline board and reporting pain below the patella on a 100mm VAS (end tags were no pain (0) and worst pain ever (100)) (25). The VISA-P, a questionnaire that indexes the severity of patellar tendinopathy, is scored out of 100 points, with a higher score meaning fewer symptoms and lower score indicating dysfunction and pain (26).

Loading/Training:

Dance teachers reported on how many hours of class per day each grade was participating in for each year of the study. Individual participants answered a questionnaire at each data collection describing time-loss and reasons for any days missed in the previous week and 6 months, as well as any injuries and modifications to class if they were not participating fully (ie: not jumping). They were also asked how the previous week of activity (dance and other physical activity) compared to

the previous 3 months of activity and rated it from "very much less activity" to "very much more activity" on a Likert scale of -4 through +4, with 0 being "same activity".

Statistical Analysis:

Descriptive statistics and linear regressions were analyzed in the R statistical package. Comparisons between normal and abnormal group features were tested using the Mann-Whitney-Wilcoxon test. Significance was determined at the 5% level. In interaction effect was tested between the normal and pathological groups regressing echo-type composition (combined types III+IV) by time.

Results

61 participants were recruited for this study, four were excluded because of missing data. Of 57 participants, 34 were female and 23 male, with an age range at baseline of 11-18 years. 29 participants had a history of injuries ranging from back pain to ankle sprains within the previous year.

Hypoechoic Area/Abnormal Group:

At the start of the study, no participants had pathology on their tendons. Over the course of 2 years, 5 of 57 participants (9%) developed hypoechoic areas during at least 2 time periods and were considered to have tendon pathology. In this pathology group three were male and two were female. The baseline age for the participants who developed pathology were 13, 15, 18, 12 and 13 years old (participants A, B, C, D, E respectively).

Maturity Offset and Peak Height Velocity:

There was no difference in the maturity offset between the normal and pathology groups at baseline or throughout the study (Wilcoxon test p=0.79 at baseline and p=0.97 throughout the study) (Table I). Within the normal group 24 female participants were pre- peak height velocity (PHV), 8 were post-PHV at baseline. By the end of the study, 9 females were still pre-PHV. Of the male participants 12 were pre-PHV, 8 were post-PHV at baseline, by the end of the study only 1 remained pre-PHV.

For the pathology group, two were post-PHV the entire study (Participants B and C). Two participants crossed peak height velocity during the study (Participant A between the third and fourth time period and Participant E between the fourth and fifth time period). While one participant remained pre-PHV for the entire study (Participant D) (Figure 1).

Greyscale Score:

At the start of the study, there was no difference in between the greyscale scores of the normal and pathology group (Wilcoxon test p=0.11). For the normal participants, prior to peak height velocity there is a high degree of variability in total greyscale scores, however after PHV, they have higher scores (5 and 6), which are consistent with mature tendon scores (11). For the abnormal group, two participants had immature tendon appearances prior to hypoechoic areas while three had more mature tendon appearances prior to developing hypoechoic areas.

Ultrasound Tissue Characterization:

At the start of the study, there were no differences in the composition of echopattern between the normal and pathology groups (Wilcoxon test: type I-IV p=0.59, p=0.37, p=0.29, and p=0.33 respectively). There was, however, a difference

in type III and IV disorganized tissue between the groups throughout the study (Wilcoxon test: p=0.001 for type III and p=0.01 for type IV). Towards the end of the study (fourth time period), there was an increase in disorganized echo-type III+IV, combined, of 4.1% for the whole cohort (p<.01), however the abnormal group showed an even greater increase of 13.6% more type III+IV over time (p<.01, Tables I and II).

Pain

Three out of five participants with pathology had no pain during the study and two developed moderate pain during the study. At baseline and follow-up, there was no difference in the VISA-P scores between the normal and pathology groups (Wilcoxon test p=0.87 at the start and p=0.74 throughout the study). Participants who had normal tendons had very little pain throughout the study; the mean VISA-P score was above a 90 at each time point, which is considered asymptomatic. Between 4-14% scored below 80 at the different time points (Table III).

There was no difference in reports of pain on the single leg decline squat at the start of the study (p=0.33), however pain trended higher during the study in the pathology group (p=0.055). Three of the pathology group participants reported no pain on the SLDS during the study while two reported moderate knee pain (the same two participants that developed pain on the VISA-P). The mean SLDS pain score was 4.8 (SD = 13.0) for the normal group and 11.4 for the abnormal group (SD=19.0).

Loading/Training

There was no difference in the reporting of jumping participation for the previous week, 6 months, or on the Likert scale for the two groups (p=0.44, p=0.88 and p=0.28 respectively).

Discussion

This longitudinal study is the first to show the development of pathology in the proximal patellar tendon during early or mid adolescence. Elite dancers as young as 12 years old, who started with structurally normal tendons, went on to develop hypoechoic areas within their patellar tendons over this 2 year period. During this longitudinal study, 9% of adolescent ballet dancers developed pathology, which is a similar prevalence to that of previous studies in the adult ballet population (12%) (3). It is possible that this incidence rate of pathology may be higher as a proportion of participants in this study had not passed PHV when data collection was completed. These findings are important as they show that patellar tendon pathology can develop during adolescence.

Discovering when and how pathology develops in ballet dancers is important and may have implications for other jumping athletes. Sports, such as rugby, volleyball and basketball, have reported a higher prevalence of tendon pathology in both symptomatic and asymptomatic junior athletes; 29% of junior volley players (10), 26% of junior basketball players (4), 29% of rugby players (27) and 32% of asymptomatic basketball players had hypoechoic areas on ultrasound (28). These findings are consistent with the main hypothesis that pathology could develop during adolescence. In this study pathology developed throughout adolescence,

both prior to and after the adolescent growth spurt, and when the tendon attachment appeared both immature and mature.

The condition of pain and dysfunction at the distal attachment of the patellar tendon in adolescent athletes is known as Osgood-Schlatter Disease (OSD). The pathological process was initially thought to be due to fragmentation of the anterior aspect of the bony tibial tuberosity. However, it is now understood to be part of normal development and can be seen in both symptomatic and asymptomatic adolescent athletes (12, 13, 29-32). Currently, development of a hypoechoic area at the proximal tendon is considered pathological, and is seen in both symptomatic and asymptomatic adolescent athletes (33).

Once present, a hypoechoic lesion may remain for an athlete's career, though it does not mean the athlete will go on to develop symptoms (3, 34). Pathology is considered to be a risk factor for developing symptoms and if it develops during adolescence, the athlete will be at a higher risk of onset of pain in adulthood compared to those with no tendon pathology. Because pathology can develop during adolescence, prevention strategies should target this developmental stage. If the development of pathology in adolescents can be reduced, the risk of developing symptoms in adulthood will also be reduced.

A hypoechoic area is only one of several risk factors associated with the onset of symptoms; other factors to be considered include loading volume. The secondary hypothesis was that the normal and pathology participants would differ in load volume and pain scores. There were few differences between the two groups, likely due to the small sample size or heterogeneity in the age and maturity offset of the cohorts. Patellar tendon pathology etiology is likely multifactorial and further research into contributing factors with larger samples, ideally in higher jumping sports like basketball or volleyball, is needed to better understand how to prevent it.

It is worth noting that while five dancers developed pathology, they did not report high degrees of functional limitation in either the VISA-P or modifications to dance participation during the study. They did have higher reports of knee pain on the single leg decline squat however this was still a fairly low report of pain. Other studies have also demonstrated that the presence of pathology does not necessarily equal pain or cause functional limitations (3, 35) and this study reinforces those findings.

Limitations:

The high degree of heterogeneity of the sample was a limitation as not all the participants started pre-PHV and ended post-PHV. There were also limitations to the UTC not being validated in human tendons, however the UTC is capable of detecting subtle changes in tendon stability within-person due to its excellent intra-rater reliability, so for the purposes of this study it was an ideal tool to observe tendon imaging changes over time with a semi-quantitative modality. Also, patellar tendinopathy and pathology are seen at lower rates in ballet versus other jumping sports. It may be beneficial repeating this study in other sports where the pathology rate is higher, such as basketball and volleyball. However, for this study it was useful

to use ballet athletes as they have a very low attrition rate in adolescence and have a regular dance schedule, aiding the tracking of loading volume.

Perspectives:

This study clarifies the question of when proximal patellar tendon pathology develops, demonstrating that it is a disease process of adolescence and perhaps prevention studies should focus on the early adolescent stage. In this study pathology was seen to develop in 9% of elite adolescent ballet dancers who started with normal tendon appearances. This is similar to the rate of pathology observed in adult elite ballet dancers (3). There was no clear association between the timing of pathology development and maturity offset or adolescent growth spurt in the pathology group. Nor were there differences between the two groups in the timing of growth spurt and loading volume. The presence of a hypoechoic area did not clearly correlate with pain. The development of tendon pathology is likely multifactorial and further research is needed to identify contributing factors.

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Tables and Figures:

Table I: Outcomes Measures for Normal and Abnormal Groups

	Start of Study					Throughout Study				
	NORMAL		ABNORMAL			NORMAL		ABNORMAL		
Outcome:	Mean	SD	Mean	SD	Wilcoxon	Mean	SD	Mean	SD	Wilcoxon
Maturity Offset	-0.38	1.33	-0.24	2.11	0.79	0.09	1.18	0.29	1.76	0.97
Echotype I	0.63	0.12	0.68	0.11	0.59	0.62	0.10	0.63	0.10	0.18
Echotype II	0.36	0.11	0.30	0.10	0.37	0.35	0.10	0.33	0.08	0.47
Echotype III	0.01	0.01	0.01	0.01	0.29	0.02	0.02	0.03	0.04	0.00*
Echotype IV	0.00	0.00	0.01	0.01	0.33	0.01	0.01	0.01	0.02	0.01*
Greyscale Score	3.87	1.56	2.40	2.30	0.11	3.94	1.72	X	X	X
VISA-P	96.14	10.26	80.00	44.72	0.87	94.45	10.95	90.17	22.04	0.74
SLDS	4.54	11.18	0.00	0.00	0.33	4.79	12.99	11.42	19.03	0.05*
Jump-week	0.08	0.27	0.00	0.00	0.54	0.14	0.35	0.20	0.41	0.44
Jump-6mo	0.10	0.30	0.20	0.45	0.49	0.27	0.44	0.28	0.46	0.88
Likert scale	-0.49	1.44	0.20	1.48	0.28	-0.35	1.37	-0.09	1.78	0.25

Table II: Changes in Type III+IV Echo-type Over Time (Interaction effect)

Type III+IV Echopattern Changes Overtime			
Time Period:		Main Effect of Time on Echotype III+IV for All Participants:	Differential Effect of Time on Echotype III+IV for Abnormal Participants:
2		-0.0002	0.001
	sd	-0.006	-0.018
3		0.003	0.003
	sd	-0.005	-0.017
4		0.041***	0.136***
	sd	-0.005	-0.017
5		0.036***	0.050**
	sd	-0.005	-0.02
Notes:			*****Significant at the 1 percent level.**
			****Significant at the 5 percent level.**

Table III: Number of participants with VISA-P scores <80 at each time period:

	Time Period:				
	1	**2**	**3**	**4**	**5**
Test and Group:					
# of participants with VISA-P under 80 in Normal group (n=52)	4	5	7	5	2
# of participants with VISA-P under 80 in Pathological (n=5)	1	0	1	0	1
Median VISA-P Normal Group	96.14	93.64	94.38	93	95
Median VISA-P Pathology Group	80	93	92	98.6	86.5
Median SLDS Normal Group	4.5	4.6	6.5	5.6	2.4
Median SLDS Pathology Group	0	15.6	19.4	3.8	20

Figure I: Changes in Maturity Offset Over Time for Abnormal Participants and Presence of Hypoechoic Area