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Mechanical properties of the patellar tendon in elite volleyball players with and without patellar tendinopathy

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ABSTRACT

Background Although differences in mechanical properties between symptomatic and healthy tendons have been observed for the Achilles tendon, the impact of tendinopathy on patellar tendon mechanics is not fully documented. The aim of the present case–control study was to assess the mechanical properties of the tendon and jump performance in elite athletes with and without patellar tendinopathy.

Methods We identified 17 male volleyball players with patellar tendinopathy and 18 healthy matched controls from a 5-year prospective cohort study on junior elite volleyball players. Outcome variables included three measures of maximal vertical jump performance and ultrasound-based assessments of patellar tendon cross-sectional area, stiffness and Young's modulus.

Results The proximal cross-sectional area of the patellar tendon was significantly larger in the tendinopathic group (133±11 vs 112±9 mm^2, respectively; p<0.001). Pathological tendons presented lower stiffness (2254±280 vs 2826±603 N/mm, respectively; p=0.006) and Young's modulus (0.99 ±0.16 vs 1.17±0.25 GPa, respectively; p=0.04) than healthy tendons. However, the difference between the countermovement jump height and the squat jump height (3.4±2.2 vs 1.2±1.5 cm, p=0.005) was significantly higher in the tendinopathic group compared with the control group.

Conclusions Patellar tendinopathy is associated with a decrease in the mechanical and material properties of the tendon in elite athletes subjected to a high volume of jumping activity. However, compared with their healthy counterparts, tendinopathic volleyball players have a better ability to utilise the stretch-shortening cycle when jumping.

INTRODUCTION

Patellar tendinopathy (jumper's knee) is a common overuse injury in sports with great demands on rapid force development in the leg extensors. An estimated prevalence as high as 45% is reported in elite volleyball players,[1] and the condition is known to be a contributing factor to early retirement.[2] Several new treatment strategies exist, for example, eccentric loading, heavy slow resistance training, sclerosing injections and injections of platelet-rich plasma.[3–6] Nonetheless, patients rarely recover completely, and the understanding of the underlying mechanisms for efficacy of treatment is limited.[3 6 7]

Training and match exposure,[8] competing at an elite level[1] compared to a non-elite level[9] and sex (men being more prone to disorder),[1 9] are significant risk factors for developing patellar tendinopathy. Incidentally, it has been shown in both a case–control study[10] and a cross-sectional study[11] that jump performance is superior in athletes with patellar tendinopathy compared to healthy athletes (the jumper's knee paradox). These findings have recently been corroborated in a 5-year prospective cohort study, where adolescent volleyball players who went on to develop tendinopathy jumped higher in a countermovement jump (CMJ) compared to players who remained healthy.[12] Yet, a higher jumping ability in individuals presenting a degenerative disorder in their tendon seems counterintuitive, in regard to the positive correlations between jump performance and the stiffness of healthy patellar tendons.[13]

Tendinopathy is related to structural tissue changes, such as increased type III collagen content,[14–17] which may adversely influence the mechanical and material properties of the tendon.[18 19] In patients with Achilles tendinopathy, greater strain,[20 21] lower stiffness[20 22] and Young's modulus, and a greater cross-sectional area (CSA)[20] have been observed in comparison with their healthy counterparts. However, such differences were not detected in the patellar tendon of recreationally active patients[4 5] or in badminton players with tendinopathy.[23] Until now, there are no data on tendon properties in patients involved in sports with a high volume of jumping activity (eg, volleyball).

Therefore, the aim of this study was to compare the mechanical and material properties of the patellar tendon between male volleyball players with tendinopathy and a group of matched controls. To minimise recruitment bias, players were included from a 5-year prospective cohort study on young, elite volleyball players. We also wanted to investigate whether the greater jump height observed in CMJ in players with tendinopathy is present in a more sport-specific spike jump test.[24 25] We hypothesised that, in spite of greater jumping ability, the patellar tendon of elite volleyball players with tendinopathy has lower stiffness and modulus than healthy controls.

MATERIAL AND METHODS

Recruitment and inclusion

The participants were former students at Toppvolley Norge, a boarding school which combines an elite volleyball development programme with a 3-year baccalaureate degree. They were recruited from a 5-year prospective cohort study, where 22 of 69 male

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volleyball players developed jumper's knee (figure 1).[12] To be included, they also had to be competing at the national elite level at the time of inclusion for the present study. Among the 22 players with jumper's knee, 20 were still playing volleyball and we matched them with 20 players from a group of 46 healthy males with respect to age, height, weight and year of inclusion into the prospective cohort study. In the jumper's knee group, three players were excluded, two declined the invitation to participate and one no longer played at the elite level. In the control group, two players no longer played at the elite level. Thus, we included 17 players with a history of jumper's knee and 18 healthy controls for further measurements. Owing to the higher prevalence of patellar tendinopathy in men compared to women,[12] we only recruited male players.

Player groups

In the original cohort study, tendinopathy was diagnosed based on the following criteria: (1) tenderness on palpation of the proximal patellar tendon or the distal part of the quadriceps tendon insertion; (2) pain during volleyball training, consistent with the tender area[1 11 12 26] and (3) hypoechoic areas and increased tendon thickness corresponding to the painful area examined by ultrasonography.[27 28] To be classified as jumper's knee, symptoms had to have persisted for at least 12 weeks and represent a substantial problem to the player.[12] Three of the originally healthy controls subsequently developed patellar tendinopathy, between the time of the cohort study[12] and inclusion into the present study, and their results are presented separately as the control–patellar tendinopathy group. Of the 17 players with tendinopathy, 1 was completely free of symptoms at the time of inclusion into the present study. Thus, we divided the participants into four groups based on their knee status at the time of the study (figure 1): (1) participants with current patellar tendinopathy (n=13), (2) participants with current quadriceps tendinopathy (n=3), (3) healthy controls (n=15) and (4) controls with current patellar tendinopathy (n=3). Participants' characteristics are shown in tables 1 and 2.

Experimental procedures

Participants underwent familiarisation with all relevant tests at least 24 h prior to data collection. Data were collected in the same order as described below and all data collection was conducted by the same investigator throughout the study period (October 2011–June 2012) at the laboratory at the Norwegian School of Sport Sciences and the laboratory at the Norwegian Olympic Sports Center (Olympiatoppen) in Oslo, Norway. Before data collection, participants did a standardised warm-up of 5–10 min submaximal treadmill running with increasing speed, starting at a moderate jogging speed individualised for each participant, followed by three submaximal 40 m sprints separated by approximately 3 min of rest.

Jump performance

Participants performed squat jumps (SJs) and CMJ (sampling rate 2 kHz) with arms akimbo on a force platform (AMTI OR6-5-1, AMTI, Watertown, Massachusetts, USA). For SJ, participants were instructed to squat until their knee joint angle reached approximately 90°, while the trunk was maintained in an upright position. One to 2 s after reaching this position, the investigator (CH) gave the signal to perform a maximal vertical jump. The highest jump out of three to five attempts was used for further analysis. CMJ was conducted in a manner similar to SJ, although the hip, knee and ankle flexions were performed from an upright position to a self-determined depth, followed by an immediate maximal vertical jump. Thereafter, the participants conducted a series (3–5) of spike jumps[24 25] under a

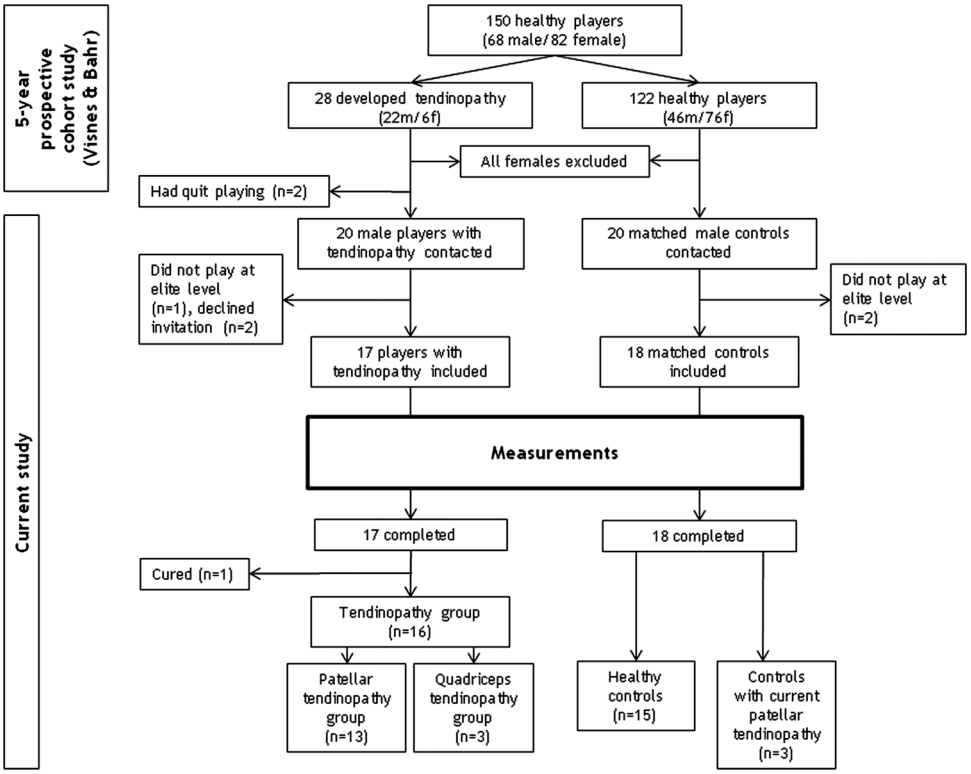


Figure 1 Flow chart depicting the recruitment and inclusion of participants into the study.

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Table 1 Participants' characteristics for the patellar tendinopathy group, quadriceps tendinopathy group, control group and control–patellar tendinopathy group

	Patellar tendinopathy (n=13)	Control (n=15)	Quadriceps tendinopathy (n=3)	Control–patellar tendinopathy (n=3)
Age (years)	20 (19–21)	21 (18–22)	22 (20–24)	21 (20–22)
Height (cm)	189 (185–192)	190 (188–191)	194 (190–197)	194 (190–198)
Weight (kg)	81 (78–85)	85 (81–89)	86 (84–88)	89 (82–95)
Patellar tendon length (mm)	53 (51–56)	52 (49–54)	52 (50–54)	56 (52–60)
VISAp score	76 (64–87)*	98 (96–100)	86 (77–94)	73 (47–98)

*Significantly different from the control group, p<0.05.
VISAp, Victorian Institute of Sport Assessment questionnaire for patellar tendinopathy.

custom-made premeasured frame: participants were instructed to use an arm swing and a classic, volleyball-specific, three-step approach to jump vertically, touching an overhead target (a red plastic wire) with their dominant hand. The target was incrementally raised by 2–5 cm between jumps until the maximum jump height was reached. Participants had a resting period of 1–3 min between each jump, and 3–5 min between each jump type. Finally, we measured the standing reach height with a tape, measured from the floor to the fingertip of the dominant hand. Participants had a rest period of approximately 15 min before proceeding with measurements of tendon properties and a peak rate of force development.

Tendon properties and peak rate of force development

For measurements of tendon properties and maximal voluntary knee extensor contractions (MVCs), the participants were secured on an isometric knee extension chair (Knee extension, Gym2000, Geithus, Norway) instrumented with a load cell (U2A, Hottinger Baldwin Messtechnik GmbH, Darmstadt, Germany). Since tendinopathy symptoms were observed on the right leg of a majority of players (except 3 cases), all tests were conducted on this side in the control group. The knee and hip angle were set at 90° (figure 2).

To assess the maximal rate of force development, participants performed three MVCs of the knee extensor muscles, with the instruction to reach their maximum force as fast as possible. Data were stored on a separate computer with a sampling rate at 1000 Hz (LabVIEW V.8.2, National Instruments Corporation, Austin, Texas, USA). Contractions were separated by a 60 s resting period. Subsequently, two MVC knee flexions were performed to enable assessment of hamstring coactivation.[29] The mechanical properties of the tendon were assessed by measuring the elongation of the patellar tendon during isometric ramp contractions. An ultrasound linear array transducer (50 mm, 5–12 MHz HD11XE, Phillips, Bothell, Washington, USA) was attached anteriorly to the patellar tendon with a custom-made device (figure 2), and the participants performed at least three maximal ramp contractions while ultrasound video sequences were recorded. A visual feedback displayed in front of the participants ensured that all ramp contractions were performed at a constant loading rate (100 N/s). Trials were discarded when the force trace deviated from the required linear pattern upon visual inspection. To limit the influence of creep on tendon loading, three submaximal preconditioning contractions were performed a few seconds prior to each ramp contraction.

Electromyographic activity of the biceps femoris muscle was recorded wirelessly (TeleMyo 2400 G2 Telemetry System, Noraxon Inc, Scottsdale, Arizona, USA) during isometric knee extensions and flexions to estimate the coactivation level. The skin over the biceps femoris was shaved and rubbed with alcohol to reduce skin impedance. Self-adhesive surface electrodes (Ambu, Blue Sensor M, Ballerup, Denmark) were attached over the muscle belly with an interelectrode distance of 2 cm. A reference electrode was placed on the patella. A wireless receiver (Mini-Receiver for TeleMyo G2, Noraxon Inc, Scottsdale, Arizona, USA) synchronised force, electromyogram (EMG) and ultrasound video.

Tendon and muscle morphology

Cross-sectional ultrasound images of the proximal, middle and distal parts of the tendon were recorded at rest and with 90° flexion of the knee. Tendon CSA was assessed with a video and image analysis software (ImageJ V.1.45s, National Institute of Health, Austin, Texas, USA). Tendon length was measured in the sagittal plane from ultrasound images, as the distance between the patellar apex and the tibial tuberosity.

Diagnosis

We palpated the patellar and quadriceps tendon insertions and obtained diagnostic ultrasound images of the patellar tendon, where hypoechoic areas and increased tendon thickness were noted. Body height was measured, and participants completed a Norwegian translation of the Victorian Institute of Sport Assessment questionnaire[30] for patellar tendinopathy (VISAp), without any usage instructions from the investigator. The VISAp form is a commonly used questionnaire for measuring pain and function in the patellar or quadriceps tendons, where 100 represents a perfectly healthy knee and 0 represents a completely non-functional, painful knee.

Table 2 Number of participants in the volleyball-specific playing positions

Playing positions	Patellar tendinopathy (n=13)	Quadriceps tendinopathy (n=3)	Control (n=15)	Control–patellar tendinopathy (n=3)
Outside hitter	5	2	8	2
Setter	3	1	3	1
Middle blocker	4	–	3	–
Libero	1	–	1	–

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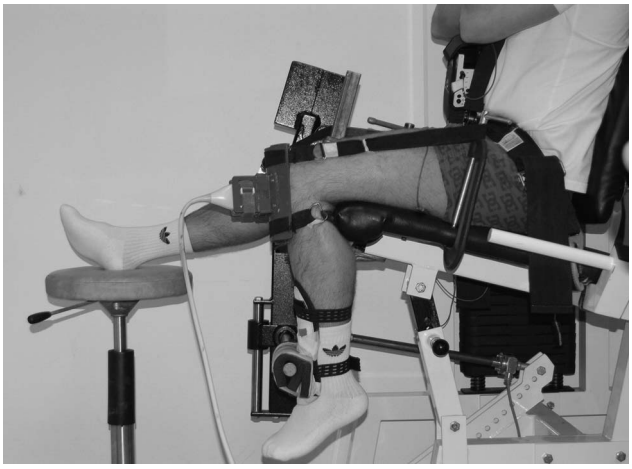


Figure 2 Measurements of tendon elongation and tendon force in an isometric knee extension chair, with a fixed hip and knee angle at 90°. The leg cuff is attached to the lower leg and the ultrasound probe firmly attached to the patellar tendon.

Data analysis

Jump performance

The SJ and CMJ results were stored and analysed on a separate computer in a custom-made Matlab-based software (Biojump V.2.4, Biomekanikk AS, Oslo, Norway). Jump height was calculated from the time integration of the vertical force.[13] The highest jumps of both SJ and CMJ were used for further analysis. The difference between CMJ and SJ was calculated to look at the participant's ability to utilise the stretch shortening cycle (SSC).[13 31] The spike jump height was calculated as the difference between standing reach height and jumping reach height.

Tendon properties

Tendon elongation was measured with video analysis software (Tracker Video Analysis and Modeling Tool V.4.62, Open Source Physics, Aptos, California, USA). The coordinates of the patella apex and the edge of the tibial plateau were tracked during the ramp contractions and changes in the distance between these references were assimilated to changes in tendon length.[32] Tendon force was calculated from the net force applied to the load cell (sampling rate 1500 Hz) corrected for antagonist coactivation,[33] external moment arm and internal moment arm. Femur length was calculated from body height,[34] and was used to estimate the internal knee joint moment arm.[35] All EMG signals were quantified by calculating the root mean square over a 50 min time period. A linear relation between force and EMG amplitude was assumed to correct for hamstring coactivation.[36] Hence, patellar tendon force (F_t) was calculated from the following equation:

$$F_t = ((F_q + F_h)M_e)/M_i$$

where, F_q is force recorded at the load cell, F_h the estimated hamstring coactivation force, M_e and M_i the external and internal moment arms, respectively.

Force-elongation curves were fitted with a second order or third order polynomial, with $R^2>0.95$, and stiffness was calculated in the final 10% of the linear part of the curve.[37] Absolute stiffness, measured at the maximal force level of each individual, and relative stiffness, measured at the highest common force level (4000 N), were calculated. We estimated Young's modulus from both absolute and relative stiffness by multiplying stiffness with the ratio between tendon length and the middle CSA. One participant was excluded from the analysis of relative stiffness and relative Young's modulus because his maximal force did not reach 4000 N. Finally, stress was obtained by dividing maximal tendon force by the middle CSA, and strain by dividing maximal tendon elongation by the resting tendon length.

Rate of force development

The rate of force development data were smoothed with running average (20 min) and the peak rate of force development (N/s) calculations were derived between 10 min (10th min–1thmin).

Statistical analyses

We analysed differences between groups with a two-tailed unpaired t test, where an α level of 0.05 was set as a significant difference. We analysed tendon CSA with a two-way analysis of variance (ANOVA) test, and with a Bonferroni post hoc test correction in case of significant interaction effects. All statistical analyses were conducted in Excel (Microsoft Office Excel 2007 Inc, Microsoft) or GraphPad Prism (GraphPad Prism V.5.00, GraphPad Software, San Diego, California, USA). Unless otherwise noted, all analyses are performed on the patellar tendinopathy group (n=13) and the control group (n=15). Data from the control–patellar tendinopathy group and the quadriceps tendinopathy group are presented separately to show the complete dataset. Data are presented as means with 95% CI unless otherwise noted.

RESULTS

Mechanical and material properties

Absolute and relative patellar tendon stiffness and Young's modulus were significantly lower in the patellar tendinopathy group compared to controls (32%, 20%, 29% and 15%, respectively; figure 3). Stiffness and Young's modulus for formerly healthy participants with current patellar tendinopathy were similar to that for symptomatic participants for both relative and absolute values. Participants with quadriceps tendinopathy had similar values compared to participants in the control group for both relative and absolute stiffness and Young's modulus. Force, stiffness, stress, strain, elongation and Young's modulus values for the patellar tendon for the patellar tendinopathy and control groups are shown in table 3.

Tendon proximal CSA was 19% larger in the patellar tendinopathy group than in the control group (figure 4).

Jump performance

The patellar tendinopathy group had a significantly greater (183%) CMJ–SJ difference compared to the controls (figure 5). There were no differences in spike jump, SJ and CMJ performance between the two groups (table 4); however, when data from the quadriceps tendinopathy group (n=3) were combined with those of the patellar tendinopathy group (n=16), there was a significant difference in the spike jump height (7 cm, 0.07–14) and spike jump reach (6 cm, 0.5–11) compared to controls.

Isometric rate of force development

There were no significant differences in the peak rate of force development between the patellar tendinopathy and control groups (figure 6).

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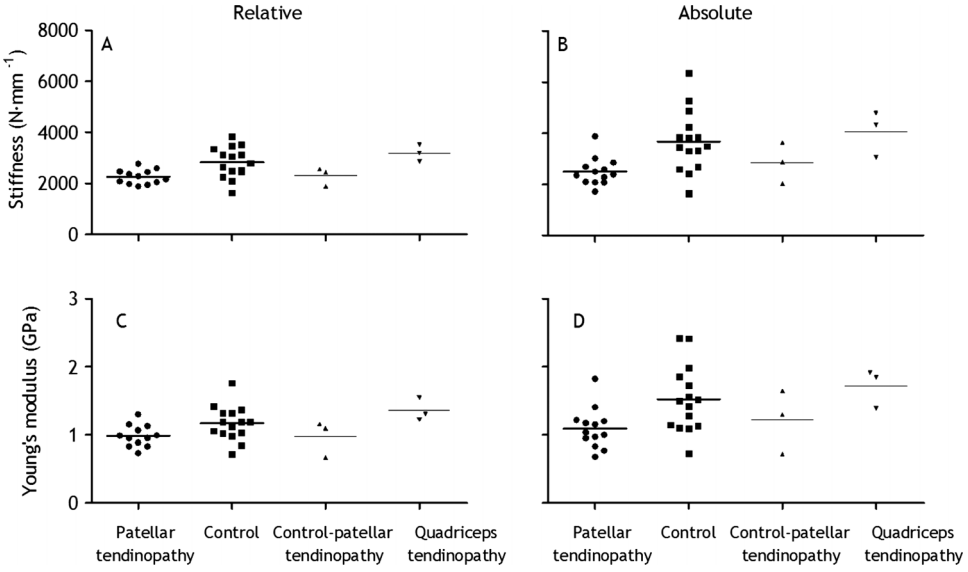


Figure 3 Image showing stiffness (A and B) and Young's modulus (C and D) for the patellar tendinopathy group (n=12 and 13 for the relative and absolute values, respectively), control group (n=15), formerly healthy participants with current patellar tendinopathy (n=3) and the quadriceps tendinopathy group (n=3). Values are presented in both relative (A and C) and absolute (B and D) values. The horizontal lines represent the group means.

DISCUSSION

This is the first report of lower stiffness and Young's modulus in symptomatic patellar tendons. In line with previous findings, the present findings confirm the larger proximal CSA of the patellar tendon with tendinopathy. These findings indicate that chronic tendinopathy is associated with altered morphological, mechanical and material properties.

Our findings support previous observations on the Achilles tendon with respect to CSA, stiffness and Young's modulus,[20 22] but are in contrast to previous findings on the patellar tendon.[4 5 23] Yet, a trend towards lower stiffness (8%) had been reported when comparing a tendinopathic group (n=8) with an age-matched and activity-matched group of healthy (n=9) recreational athletes.[5] It should also be noted that the present protocol differs from previous studies on patellar tendinopathies,[4] where the age range of patients was as large as 18–50 years, and where comparisons were made within individuals, between those with tendinopathic and healthy tendons. Age and training type have previously been shown to potentially influence the properties of the patellar tendon.[37–39] These parameters could explain the differences between the present findings and earlier studies, where they may have induced a larger variability. In contrast, we examined a group of elite athletes practicing the same physical activity, within a more homogenous age group, and compared these injured individuals with the healthy control group.

Although we found a lower stiffness in the symptomatic tendons, the tendon proximal CSA was larger in these tendons compared to controls, in accordance with the previous findings.[26] Theoretically, a greater CSA would have led to a larger stiffness if the tendon composition were the same. However, an ultrasound examination revealed that the thickened area was structurally abnormal, with substantial collagen disarray and hypoechoic areas reflecting increased ground substance.[26 40 41] Therefore, the present data suggest that tissue degeneration impacts both the mechanical and material properties of the patellar tendon.

One strength of this study lies in the fact that all participants were recruited from a 5-year cohort study,[12] following a population of adolescent athletes prospectively to include 17 of 19 patients and 18 of 18 controls among those eligible (still competing at a high level), unlike other studies where bias may have occurred in the selection process.[4 5 23] However, tendon properties were not measured at baseline. Therefore, we can only speculate as to whether the differences in mechanical and material properties observed are the cause or the consequence of chronic tendinopathy.

In line with previous findings,[10–12] symptomatic players had an overall better jump performance compared to matched controls, as evidenced by a greater difference between CMJ and SJ height and by the sport-specific spike jump. The difference

Table 3 Mechanical and material properties for the patellar tendinopathy and control groups

	Patellar tendinopathy (n=13)	Control (n=15)	95% CI	p Value
Peak force (N)	5298 (4593–6002)	5809 (5195–6423)	511.4 (−464.2 to 1487)	0.29
Normalised peak force (N/BW$^{0.67}$)	278 (244–313)	295 (268–322)	16.8 (−29 to 62)	0.45
Stiffness-absolute (N/mm)	2504 (2209–2800)	3684 (3082–4287)	1180 (441 to 1919)	0.003
Stiffness-relative (N/mm)	2254 (2096–2412)	2826 (2521–3131)	571.6 (182 to 961)	0.006
Stress (Mpa)	43 (37–50)	47 (43–51)	3.4 (−5 to 11)	0.39
Strain (%)	7 (6–7)	7 (6–8)	0.2 (−0.73 to 1.07)	0.70
Elongation (mm)	4 (3–4)	4 (3–4)	0.04 (−0.48 to 0.57)	0.87
Young's modulus absolute (Gpa)	1.09 (0.93–1.25)	1.53 (1.28–1.77)	0.4 (0.11 to 0.75)	0.01
Young's modulus relative (Gpa)	0.99 (0.89–1.08)	1.17 (1.04–1.30)	0.2 (0.01 to 0.36)	0.04

Stiffness absolute and Young's modulus absolute are absolute values calculated from peak force. Stiffness relative and Young's modulus relative are values calculated from the greatest common force level at 4000 N (n=12).

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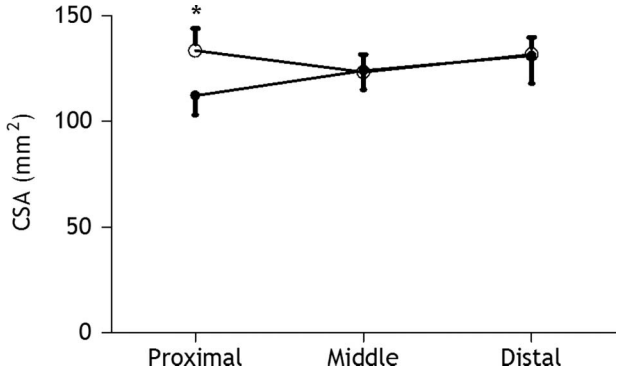


Figure 4 Image showing the tendon cross-sectional area (CSA) for the patellar tendinopathy group (open figures; n=13) and control group (closed figures; n=15 (distal CSA, n=13)) at the proximal, middle and distal parts of the tendon; mean±SD. *Significantly different from control, p<0.001. When comparing middle CSA with the same controls used for distal CSA (n=13) to the patellar tendinopathy group (n=13), no significant difference was found (p=0.92).

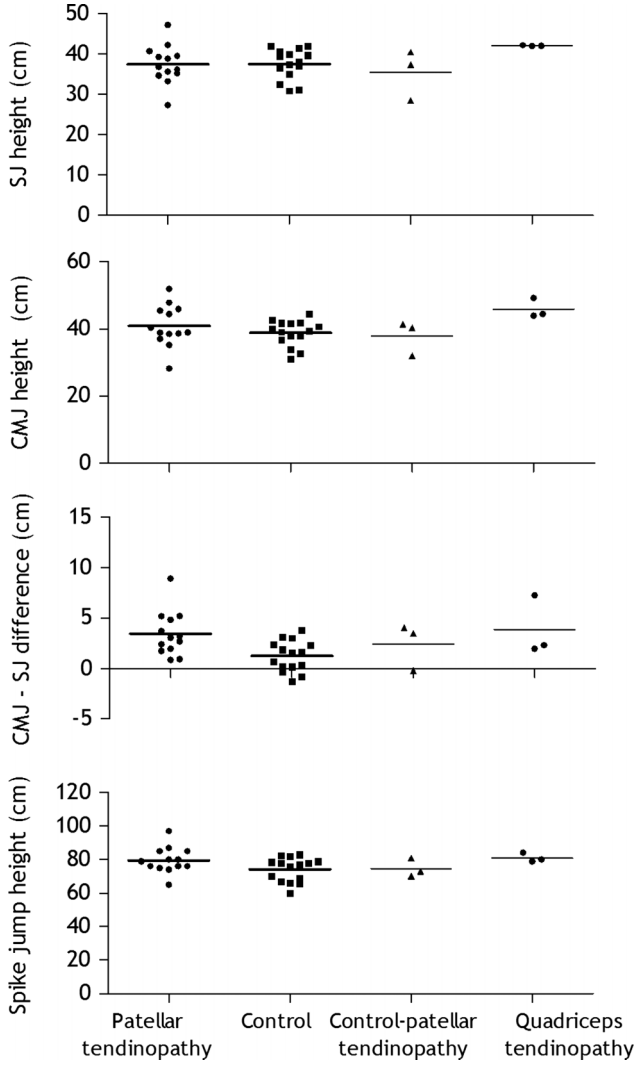


Figure 5 Image showing the SJ (squat jump) height (A), CMJ (countermovement jump) height (B), CMJ–SJ difference (C) and spike jump height (D) for the patellar tendinopathy group (n=13), control group (n=15), formerly healthy participants with current patellar tendinopathy (n=3) and quadriceps tendinopathy groups (n=3). Horizontal lines represent the group means.

Table 4 Jump performance for the patellar tendinopathy and control groups

	Patellar tendinopathy (n=13)	Control (n=15)	Difference	p Value
Standing reach (cm)	249 (244–254)	249 (246–252)	0.3 (−5.9 to 6.5)	0.92
Spike jump reach (cm)	329 (323–334)	323 (319–327)	5.8 (−1.5 to 13.1)	0.11
Spike jump height (cm)	80 (75–84)	74 (71–78)	5.5 (−0.3 to 11.3)	0.06
SJ (cm)	37 (35–40)	38 (36–39)	−0.1 (−3.5 to 3.3)	0.94
CMJ (cm)	41 (37–44)	39 (37–41)	2.1 (−1.9 to 6.0)	0.29
CMJ–SJ difference (cm)	3.4 (2–5)	1.2 (0.5–2.0)	2.2 (0.7 to 3.6)	0.004

CMJ, countermovement jump; SJ, squat jump.

between CMJ and SJ has previously been suggested to reflect an athlete's ability to utilise SSC.[13 31] In the concentric SJ, however, no differences between the two groups were observed. Consequently, players with patellar tendinopathy seem to have an increased capacity to take advantage of the elastic components of the extensor apparatus by utilising their SSC more efficiently, compared to healthy players.

Yet the higher jumping capability and the lower tendon stiffness of the present tendinopathic patients contrast with the positive correlation found previously between these variables in male cyclists and volleyball players.[13] Such relations between jump performance (SJ, CMJ and spike jump) and tendon stiffness or pathology (VISAp) were not observed in the current study for either the control group or the patellar tendinopathy group. The reasons for this discrepancy may include methodological differences, for example, different tendon testing method[13] and the different sample of participants (volleyball players and cyclists vs volleyball players only). Nevertheless, in light of these previous findings, a causal link between the patellar tendon properties and the higher jumping performance of tendinopathic volleyball players would seem highly speculative. Instead, based on previous literature suggesting that tendinopathy may result from inappropriate or excessive tendon

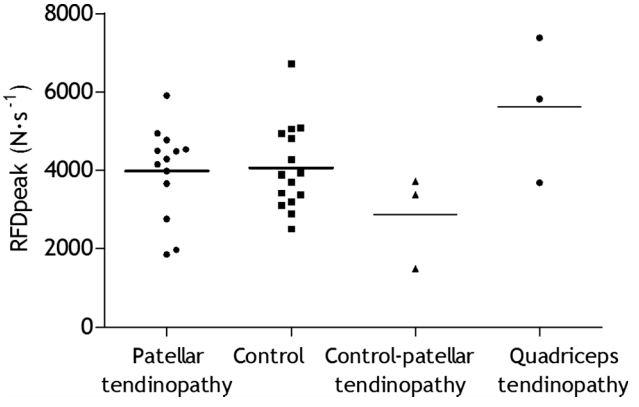


Figure 6 Image showing the isometric knee extension peak rate of force development (RFDpeak) for the patellar tendinopathy (n=13) and control (n=15) groups, formerly healthy participants with current patellar tendinopathy (n=3) and quadriceps tendinopathy groups (n=3). Horizontal lines represent group means.

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stress,[1 42–44] or different jump/landing strategies,[45 46] players who jump higher during practice and matches may be more susceptible to develop patellar tendinopathy. This is supported by the 5-year cohort study from which the present participants were recruited, in which individuals with a greater jump height developed tendinopathy.[12]

CONCLUSION

This study provides evidence of differences in tendon properties between symptomatic and asymptomatic elite volleyball players. Stiffness and Young's modulus were lower in players with patellar tendinopathy, in spite of a greater difference between CMJ and SJ height.

What are the new findings?

- Despite a larger proximal cross-sectional area, the patellar tendon of volleyball players with tendinopathy displayed lower stiffness and Young's modulus than healthy controls.
- Players with patellar tendinopathy have a better jumping ability in tasks, implying the storage and release of elastic energy.
- Symptomatic players (patellar and quadriceps tendinopathy) have a greater spike jump height than healthy players.

How might it impact on clinical practice in the near future?

This research shows that there are substantial differences in tendon and muscle properties between players with patellar tendinopathy and healthy players, suggesting that it may be possible to use these differences to identify players at risk, and consequently to adapt their training programmes to prevent the development of tendinopathy.

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Contributors CH contributed to manuscript writing, concept and design, data acquisition, data analysis and interpretation. JBM, ORS, RB and TR were involved in manuscript review/revision, concept and design, data analysis and interpretation. MMM and VJ were involved in manuscript review/revision, concept and design, and data acquisition. HV was involved in manuscript review/revision, concept and design, and data interpretation.

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