Tendinous tissue properties after short and long-term functional overload: Differences between controls, 12 weeks and 4 years of resistance training

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Tendinous tissue properties after short and long-term functional overload: Differences between controls, 12 weeks and 4 years of resistance training

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Short Title:

Tendon adaptation to functional overload

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Abstract

Aim: The potential for tendinous tissues to adapt to functional overload, especially after several years of exposure to heavy resistance training is largely unexplored. This study compared the morphological and mechanical characteristics of the patellar tendon and knee-extensor tendon-aponeurosis complex between young men exposed to long-term (4 years; n=16), short-term (12 weeks; n=15) and no (untrained controls; n=39) functional overload in the form of heavy resistance training. **Methods:** Patellar tendon cross-sectional area, vastus-lateralis aponeurosis area and quadriceps femoris volume, plus patellar tendon stiffness and Young's modulus, and tendon-aponeurosis complex stiffness, were quantified with MRI, dynamometry and ultrasonography. **Results:** As expected long-term trained had greater muscle strength and volume (+58% and +56% vs untrained, both $P<0.001$), as well as a greater aponeurosis area (+17% vs untrained, $P<0.01$), but tendon cross-sectional area (mean and regional) was not different between groups. Only long-term trained had reduced patellar tendon elongation/strain over the whole force/stress range , whilst both short-term and long-term overload groups had similarly greater stiffness/Young's modulus at high force/stress (short-term +25/22%, and long-term +17/23% vs untrained; all $P<0.05$). Tendon-aponeurosis complex stiffness was not different between groups (ANOVA, $P = 0.149$). **Conclusion:** Despite large differences in muscle strength and size, years of resistance training did not induce tendon hypertrophy. Both short-term and long-term overload, demonstrated similar increases in high force mechanical and material stiffness, but reduced elongation/strain over the whole force/stress range occurred only after years of overload, indicating a force/strain specific time-course to these adaptations.

Key Words: Aponeurosis, Hypertrophy, Muscle, Resistance Training, Stiffness, Tendon

Introduction

Tendons are integral to *in vivo* neuromechanical function transmitting skeletal muscle contractile force to the skeleton whilst also optimising the contractile conditions via their viscoelastic properties.[1,2] The response of tendinous tissue to mechanical loading is of great interest, since it may influence function[3,4,5,6] and be related to injury incidence.[7-9]. Impaired tendinous tissues properties are evident in older adults and patient groups[10-14] and are associated with reduced muscle-tendon unit functional capacity.[3,5,11,15] Functional overload, in the form of resistance exercise is widely recommended for improving musculo-skeletal function of all adults,[16,17] including older individuals and patients (e.g. osteoarthritis;[18]). However our understanding of how tendons alter their properties in response to short-term (weeks-months) and especially long-term (years) loading is limited. Tendinous tissue may exhibit morphological (cross-sectional area), mechanical (stiffness), and material (Young's modulus) adaptations to functional overload,[19,20] however the magnitude and time-course of these adaptations has not been clearly elucidated.

Whilst skeletal muscle tissue has been widely documented to undergo hypertrophy in response to functional overload with resistance training,[21] the evidence for tendon hypertrophy is equivocal; short-term resistance training studies have reported region specific increases in tendon cross-sectional area[22-24] or no change.[25-27] Explanations for this controversy could be the relatively slow turnover of collagenous tissues,[28,29] and thus changes in tendon size within the first 14 weeks of resistance training that are on the threshold of what can be accurately detected. A substantially longer exposure to functional overload may provide sufficient time for the accumulation of new tissue and thus demonstrable tendon hypertrophy. However, preliminary cross-sectional studies of long-term functional overload vs untrained controls have used insufficient methods to dispel this conflict, reporting tendon hypertrophy (low resolution ultrasound[30]) or no hypertrophy for 4 out of 5 tendon sites (limited locations along the tendon with MRI[31]). The potential for aponeurosis hypertrophy in response to resistance training has also had limited research attention.[32,33]

Short-term functional overload with resistance training (up to 14 weeks) utilising high load contractions consistently increases 'free' tendon[22,24,34,35] and tendon-aponeurosis complex [23,25,26,36-38] stiffness. The increased tendon stiffness after short-term resistance training is typically ascribed to the approximately parallel increases in tendon Young's modulus (material stiffness[24,35]) rather than substantive changes in tendon size, as mentioned above. However, the potential for further changes in tissue mechanical and material stiffness after long-term resistance training remains largely unexplored. Preliminary reports include no difference in patellar tendon material stiffness between long-term resistance trained and untrained men[30] and no additional changes in Achilles tendon-aponeurosis stiffness from 14 weeks to 18 months of resistance training in elderly women.[39] However, both these studies assessed stiffness at different forces/stresses, which confounds comparable measurements of the curvi-linear *in vivo* stress-strain relationship.[40,41]

Whilst existing data have been insufficient to confirm if functional overload results in hypertrophy of tendinous tissues, and whether mechanical and material stiffness continues to adapt with prolonged resistance training, we theorised that (i) the high tendinous tissue loads consequent to the known adaptations of large increases in muscle strength and size after long-term loading[21], and (ii) prolonged exposure to these high loads would trigger substantial adaptive responses in the tendinous tissues, in order to constrain peak tissue strain within sub-failure physiological limits[42,43]. The purpose of this study therefore was to compare the morphological and mechanical properties of the patellar tendon (stiffness, Young's modulus, CSA [mean and regional]) and quadriceps femoris tendon-aponeurosis complex (stiffness, muscle volume, vastus lateralis aponeurosis area), between participants exposed to long-term (4 years [LTT]) and short-term (12 weeks [STT]) resistance training and no (untrained controls [UC]) functional overload. Specific hypotheses were that tendon characteristics would be progressive according to the duration of overload exposure (controls<short-term<long-term), and specifically that long-term overload (resistance training) would be characterised by not only greater muscle size and strength, but also a larger tendon and aponeurosis, higher tendon Young's modulus, as well as greater free tendon and tendon-aponeurosis stiffness.

Results

Group Characteristics

Age, height and body mass were similar between UC and STT groups ($P = 0.262$, $P = 0.488$ and $P = 0.465$ respectively; Table 1), while LTT were younger, taller and had a larger body mass than UC and STT (all $P \leq 0.003$). Tendon-aponeurosis complex and patellar tendon length were similar between UC and STT ($P = 0.114$ and $P = 0.195$), although LTT had longer tissue lengths than both UC and STT (tendon-aponeurosis complex LTT +6.5% vs UC and +9.2% vs STT; both $P<0.001$; patellar tendon length LTT +9.8% vs UC $P = 0.006$, and +15.5% vs STT $P = 0.022$).

Muscle-tendon unit size and strength

Maximal voluntary torque (Figure 4a) differed between all three groups, being considerably greater in LTT than UC (+58.1%, $P < 0.001$, ES = 2.90 "very large") and STT (+34.4%, $P < 0.001$, ES = 1.66 "large"). STT was also stronger than UC (+17.6%, $P = 0.001$, ES = 1.04 "moderate"). QUADSvol (Figure 4b) was considerably larger in LTT than UC (+55.7%, $P <$

0.001, ES = 3.55 “very large”) and STT (+46.2%, P < 0.001, ES = 2.83 “very large”), although SST was similar to UC (+7%, P = 0.179, ES = 0.42 “small”). Vastus lateralis aponeurosis area (Figure 4c) was also larger in LTT than UC (+17.3%, P < 0.001, ES = 1.41 “large”) and STT (+13.5%, P = 0.006, ES = 1.09 “moderate”), but STT was not different to UC (+3.3%, P = 0.331, ES = “small”). In contrast, patellar tendon mean CSA (Figure 4d) was similar between groups (ANOVA P = 0.169), and this was also the case for regional patellar tendon CSA (proximal, middle, distal; ANOVA P > 0.141; Table 2), demonstrating no overall or region specific hypertrophy.

Patellar tendon mechanical properties (Table 3)

The patellar tendon force-elongation relationships (Figure 2a) indicated that patellar tendon elongation at the highest common force level (4200 N, Figure 2b) of LTT was 13.5% less than UC (2.6 ± 0.5 vs 3.0 ± 0.6 mm, P = 0.063, ES = 0.75 “moderate”) and 15.4% lower than STT (3.1 ± 0.6 mm, P = 0.048, ES = 0.86 “moderate”), indicating greater stiffness over the whole force range up to 4200 N for LTT only, whereas STT and UC were similar (P = 0.698, ES = 0.10 “trivial”). However, patellar tendon stiffness measured over a high force range (3360-4200N; Figure 2c) was greater for SST (+24.5%, P = 0.0004, ES = 1.27 “large”) and LTT (+16.7%, P = 0.021, ES = 0.85 “moderate”) than UC, though similar for LTT and STT (P = 0.287, ES = 0.35 “small”).

Patellar tendon stress-strain relationships (Figure 3a) revealed that at the common stress level of 40 MPa (Figure 3b), patellar tendon strain of LTT (5.1 ± 1.0%) was 24.4% lower than UC (6.4 ±1.4%, P = 0.008, ES = 1.30 “large”) and 19.9% less than STT (6.7 ± 1.7%, P = 0.006, ES = 0.97 “moderate”), indicating greater material stiffness over the whole stress range up to 40 MPa for LTT only, whereas STT and UC were very similar (P = 0.369, ES = 0.17

"trivial"). However, patellar tendon Young's modulus (Figure 3c) derived over a common stress range (32-40 MPa) was greater for both SST (+21.9%, P = 0.003, ES = 1.00 "moderate") and LTT (+23.3%, P = 0.002, ES = 1.13 "moderate") than UC, but was very similar for LTT and STT (P = 0.855, ES = 0.06 "trivial").

Tendon-aponeurosis complex mechanical properties (Table 3)

Force-elongation relationships for the tendon-aponeurosis complex (Figure 4a) showed that at the common force level of 4200 N (Figure 4b), tendon-aponeurosis complex elongation exhibited no main group effect (ANOVA, P = 0.375), indicating similar overall tendon-aponeurosis complex elongation in both resistance trained and the untrained group. Likewise, tendon-aponeurosis complex stiffness (Figure 4c) was not statistically different between groups (ANOVA, P = 0.149).

Discussion

The present study compared the morphological, mechanical and material properties of the patellar tendon and knee extensor tendon-aponeurosis complex between young men exposed to long-term (4 years), short-term (12 weeks) and no (untrained controls) functional overload. The main findings were that despite large differences in muscle strength and size, there were modest differences in aponeurosis size, greater in LTT only, and no differences in patellar tendon CSA. Only LTT had reduced elongation/strain over the whole force/stress range up to 4200 N/40 MPa, whilst both overload groups had greater patellar tendon stiffness/Young's modulus at high force/stress than UC. Therefore short-term overload appears sufficient to produce changes in high force mechanical and material stiffness, with no further adaptation with prolonged exposure, but changes in elongation/strain over the whole force range occurred only after years of overload (LTT). Contrary to these differences in tendon mechanics, tendon-aponeurosis complex stiffness and strain were similar between all three groups.

Tendon and aponeurosis size

The duration of overload showed progressive differences in muscle strength between the groups (STT +18% and LTT +58% vs UC) and muscle size was also substantially greater after regular long-term loading (LTT +56% vs UC). Despite these substantial differences, and contrary to our hypothesis there were no group differences in patellar tendon mean or regional CSA. The similar patellar tendon CSA of STT than UC might have been expected as there is contrasting evidence for tendon hypertrophy after short-term resistance training (8-14 weeks), that has been attributed to limited region-specific hypertrophy and/or slow tendon collagen turnover.[28,29] Nonetheless we hypothesised that LTT would exhibit greater tendon hypertrophy, due to a combination of (i) their higher loading and stress/strain as a consequence of their substantially greater strength, and (ii) prolonged regular exposure to 4 years of these high loads, that might provide sufficient stimulus for the disruption of tissue homeostasis and time for an accumulation of tendon collagen.[44] However there were no differences in tendon CSA between LTT and UC despite the substantial differences in muscle strength and size, indicating that tendon size does not adapt in proportion to either muscle size or strength/loading. Our study utilised MRI (regarded as the most accurate method[45,46]) to assess CSA along the full tendon length (typically 20 slices; capturing region specific CSA), and images were acquired with sensitive spatial resolution (2 mm thick images, 0 mm gap, pixel size 0.313 x 0.313 mm), as well as careful tendon segmentation performed on each image by the same-blinded investigator. Moreover, with this procedure tendon CSA measures demonstrated very good reliability (CV $\leq$ 3.5%), which provides confidence in the validity of this data. Hitherto, cross-sectional studies of tendon size in long-term vs untrained individuals present conflicting evidence from unconvincing methodologies (low resolution ultrasound[30]; limited locations along the tendon with MRI[31]). A sole longitudinal study used mixed low and high load training of older females, reporting short-term tendon hypertrophy after 14 weeks, but no further long-term (1.5 years) changes, perhaps due to the surprising lack of long-term strength improvements and thus limited overload.[39] In contrast our results provides convincing evidence that long-term (4 years) exposure to high loads (+58% greater strength and +56% greater size) is not a stimulus for tendon hypertrophy.

In support of our findings, there is evidence that functional overload via resistance training does not stimulate *in vivo* tendon collagen synthesis,[47] nor increased concentration of procollagen type 1 N-propetide, a biomarker of collagen synthesis, in the patellar tendon peritendinous tissue, concomitant with no change in tendon CSA after short-term resistance training.[27] In contrast, lower intensity higher volume loading, equivalent to endurance training, might induce *in vivo* tendon collagen synthesis; increased peritendinous tissue procollagen peptide levels,[48] and uptake of radio-labelled amino acids,[49] although this is not a consistent finding.[50,51] Furthermore long term habitual exposure to endurance training or high volumes of low-moderate loading have been found to induce greater tendon size: larger tendon CSA in distance runners vs non-runners[52,53] and in dominant vs non-dominant limbs after asymmetrical loading[54]. Therefore it may be that high volumes of low/moderate loading may be the important stimulus for tendon hypertrophy, as chronic exposure to high load does not appear to be a key stimulus.

In contrast to tendon size, LTT, but not STT, had a much larger VL aponeurosis area than UC (+17%), demonstrating that aponeurosis size is responsive to the long-term functional overload of the muscle-tendon unit via resistance training. This is coherent with the limited previous reports of greater aponeurosis size post short-term resistance training and in well-trained weightlifters vs untrained.[32,33] The greater aponeurosis area for LTT vs UC was however substantially smaller than the difference in muscle size (+17 vs +56%), which may be attributable to the greater rate of myofibrillar than connective tissue collagen synthesis in response to resistance exercise.[28,55]

Patellar Tendon Stiffness

In the absence of group differences in patellar tendon CSA, our results for patellar tendon stiffness were attributable to parallel changes in material stiffness, which is in accordance with extensive literature indicating that enhanced material properties are the primary cause of increased mechancial properties.[19,20] As expected, STT and LTT possessed greater high force patellar tendon stiffness/Young's modulus than UC, which is in accordance with previous short-term resistance training studies[22,24,30,34,35]: mean change after resistance training ~27/22% for tendon stiffness/modulus.[20] Interestingly though LTT had no greater patellar tendon stiffness/Young's modulus than STT.

However LTT did demonstrate lesser overall patellar tendon elongation/strain at a common force/stress than STT and UC, and thus greater absolute and material stiffness over the whole force range. Consequently short-term overload was sufficient to produce changes in high force stiffness/Young's modulus, with no further adaptation after prolonged exposure, but changes in elongation/strain over the whole force range occurred only after years of overload (LTT). Given the identical elongation/strain at high forces/stress after STT and LTT, the effect of lower elongation/strain over the whole force range after LTT could only be due to greater stiffness/young's modulus at lower forces/stresses. Qualitatively this difference appears to be due to greater resistance to strain at low stress levels (<10MPa), as the gradients of the stress-strain relationships after the initial most compliant region of tendon deformation were equivalent in STT and LTT. Overall, our data imply that the potential mechanisms (changes to internal structure and/or composition[56,57]) underpinning an increased high stress tendon modulus, after short-term loading, are likely saturated after 12 weeks leading to a plateau in adaptation, where as low stress-specific material adaptations appeared to continue as these were most pronounced after long-term functional overload. This stress specific time course of tendon adaptation, with decreased strain at high stresses occurring first after short-term loading, followed later by decreased strain at low-stresses after continued long-term loading is a novel finding. More detailed longitudinal investigations are required to verify these results and the mechanisms for this apparent stress specific time-course of adaptation.

The validity our findings are reinforced by the thorough measurements of elongation, stiffness and strain; e.g. multiple contractions at a standardised loading rate, duplicate measurement sessions, measurements at identical absolute forces. Representative data were derived across two sessions to yield good inter-test reliability for all stiffness, elongation and stress measurements (CV <10%). In particular our methods avoided the likely bias of higher stiffness measurements for stronger individuals due to: contracting at a higher loading rate during fixed duration ramp contractions; or measuring stiffness/young's modulus at different absolute forces (i.e. relative forces) on the curvilinear force-elongation/stress-strain relationship.

Tendon-Aponeurosis Stiffness

Surprisingly, there were no group differences in tendon-aponeurosis complex mechancial properties despite the presence of much larger muscle and aponeurosis size for LTT. This finding is in contrast to previous reports of greater knee-extensor tendon-aponeurosis complex stiffness assessed with repeated measures pre and post short-term resistance training.[26,36,37] It is possible that a cross-sectional design lacks the sensitivity to detect relatively modest differences in tendon aponeurosis stiffness. Alternatively, previous studies have commonly measured stiffness at different absolute forces pre and post training, which may have accentuated the scale of this training adaptation. The measurement of tendon-aponeurosis complex stiffness is also not considered to be as robust as that of 'free tendon'.[58] This is because it may reflect not only tendon-aponeurosis deformation, but also the active state of the muscle fibres parallel to the aponeurosis,[59] as well as the fact that single site measures of aponeurosis deformation with 2-D imaging may provide only a crude index of the stiffness of a 3-D structure. Further investigations could incorporate three-dimensional imaging techniques (ultrasound[60,61] or MRI[62,63]) that can capture the complex bi-axial deformation of the muscle and aponeurosis along the length of the tendon-aponeurosis complex.

In summary, the greater quadriceps femoris strength and volume in LTT (+58 and +56% vs UC) was associated with modest increases in vastus lateralis aponeurosis area (+17% vs UC), but not matched by a larger patellar tendon cross-sectional area, indicating that long-term functional overload via high force resistance training does not lead to extramuscular tendon hypertrophy. Short- and long-term overload groups had similar, but greater patellar tendon stiffness/Young's modulus at high force/stress than UC, but only LTT had reduced elongation/strain over the whole force/stress range up to 4200 N/ 40 MPa that was attributable to changes at lower force/stress. Therefore we found evidence for a stress specific time-course of adaptation in the patellar tendon with short-term overload sufficient to produce changes in high force mechanical and material stiffness, with no further adaptation to prolonged exposure, but increased low stress stiffness only occurring after years of overload (LTT).

Materials and Methods

Participants

Seventy young men provided written informed consent before completing this study, which was approved by the Loughborough University Ethical advisory committee, and was conducted according to the principles expressed in the Declaration of Helsinki. All participants were healthy and free from musculoskeletal injury with no previous history of tendon pathology. The untrained control group (UC, n = 39) had no lower body resistance training experience for >18 months. The short-term trained group (STT, n = 15) were measured post 12-weeks of supervised resistance training. The long-term trained (LTT, n = 16) group had 4.0 ± 0.8 (mean ± SD) years of systematic heavy-resistance training experience (~3 x wk of quadriceps sessions; typical exercises were squat, lunge, step-up, leg press). LTT participants typically reported some nutritional supplement consumption (predominantly whey protein and creatine), although none declared illegal performance-enhancing substance use.

Experimental Design

Participants visited the laboratory for a familiarisation session, (STT were familiarised pre-training) and two duplicate measurement sessions that were averaged to improve the reliability of the measurements. Participants were seated in a custom-built isometric strength-testing chair and completed a series of maximal voluntary contractions (MVCs) and ramp voluntary contractions of the knee extensors as well as knee flexor MVCs of the dominant leg (preferred kicking leg). MVCs established maximal voluntary torque (MVT) and ramp contractions were performed to permit tissue stiffness estimation. Knee joint torque was recorded throughout contractions. Knee flexor surface electromyography was recorded during knee flexor MVCs and knee extensor ramp contractions. All contractions were performed with equivalent resting joint angle configurations. Ultrasound images of the vastus lateralis and patellar tendon were recorded throughout the ramp contractions to assess tissue elongation. Measurement sessions were performed at a consistent time of the day (± 2 hours), separated by at least 2 days and started between 12:00–19:00 p.m. Participants were instructed not to participate in strenuous physical activity, consume alcohol/refrain from caffeine consumption in the 36/6 hours before measurement sessions. All participants were instructed to maintain their habitual physical activity and diet throughout the study. For the SST group, post-measurement sessions one and two took place 3-5 and 6-8 days following the last training session. Magnetic resonance imaging (MRI) was performed to assess quadriceps femoris muscle, vastus lateralis aponeurosis, and patellar tendon size. Participants were instructed to refrain from strenuous physical activity in the 24 hours prior to the MRI scan. For the STT group, MRI was conducted 2-3 days after the final training session and prior to post measurement sessions.

Short-term trained (STT) Group: Training Intervention

Training sessions were completed three times per week on the same apparatus and with equivalent joint angles as used for measurement sessions. After a brief warm-up of sub-maximal contractions of both legs, participants completed four sets of ten unilateral isometric knee-extensor contractions of each leg, with sets alternating between dominant and non-dominant legs until 4 sets per leg had been completed. Contractions were sustained at 75%MVT, with 2 s rest between each contraction. In order to control the torque rise and hold times, participants were presented with a target torque trace 2 s before every contraction and instructed to match this target, which increased torque linearly from rest to 75% MVT over 1 s before holding a plateau at 75%MVT for a further 3 s. MVCs were performed at the start of each training week to re-establish MVT and prescribe training torques.

Torque Measurement

Participants were positioned in an isometric strength-testing chair with resting knee and hip joint angles of ~115° and ~126° (180° = full extension), respectively. The resting joint angle configurations were determined from digitisation of sagittal plane video during pilot work. Adjustable straps were tightly fastened across the pelvis and shoulders to prevent extraneous movement. An ankle strap (35 mm width reinforced canvas webbing) was placed ~15% of tibial length (distance from lateral malleolus to knee joint space) above the medial malleolus, and positioned perpendicular to the tibia and in series with a calibrated S-Beam strain gauge (Force Logic, Berkshire, UK). The analogue force signal was amplified (x370; A50 amplifier, Force Logic UK) and sampled at 2,000 Hz using an A/D converter (Micro 1401; CED, Cambridge, UK) and recorded with Spike 2 computer software (CED). In offline analysis, force signals were low-pass filtered at 500 Hz using a fourth order zero-lag Butterworth filter, gravity corrected by subtracting baseline force, and multiplied by lever length, the distance from the knee joint space to the centre of the ankle strap, to calculate torque values.

Knee Flexor Electromyography (EMG)

Surface EMG recordings over the biceps femoris (BF) and semitendinosus (ST) were made with a wireless EMG system (Trigno; Delsys Inc, Boston, MA) were made during knee flexor MVCs and knee extensor ramp contractions. Following preparation of the skin (shaving, abrading and cleansing with alcohol) single differential Trigno standard EMG sensors (1 cm inter electrode distance; Delsys Inc, Boston, MA) were attached over each muscle using adhesive interfaces. Sensors were positioned parallel to the presumed frontal plane orientation of the underlying muscle fibres at 45% of thigh length (distance from the greater trochanter to the lateral knee joint space) measured from the popliteal crease. EMG signals were amplified at source (x300; 20-450 Hz bandwidth) before further amplification (overall effective gain x 909) and sampled at 2000 Hz via the same A/D converter and computer software as the force signal, to enable data synchronization. In offline analysis, EMG signals were corrected for the 48 ms delay inherent to the Trigno EMG system.

Knee Extension and Flexion Maximal Voluntary Contractions

Following a brief warm-up (3 s contractions at 50% [x3], 75% [x3] and 90% [x1] of perceived maximal), participants performed 3-4 MVCs and were instructed to either 'push as hard as possible' (knee extension) or 'pull as hard as possible' (knee flexion) for 3-5 s and rest $\geq$ 30 s. A horizontal cursor indicating the greatest torque obtained within the session was displayed for biofeedback and verbal encouragement was provided during all MVCs. The highest instantaneous torque recorded during any MVC was defined as MVT. During knee flexor MVCs EMG amplitude was calculated as the root mean square (RMS) of the filtered EMG signal of the BF and ST over a 500 ms epoch at knee flexion MVT (250 ms either side) and averaged across the two muscles to give knee flexor EMG_{MAX}.

MRI measurement of Muscle Tendon Unit Morphology and Moment Arm

T1-weighted MR (1.5 T Signa HDxt, GE) images of the dominant leg (thigh and knee) were acquired in the supine position at a knee angle of 163° (due to constraints in knee coil size) and analysed using OsiriX software (Version 6.0, Pixmeo, Geneva, Switzerland). Using a receiver 8-channel whole body coil, axial images (time of repetition/time to echo 550/14, image matrix 512 x 512, field of view 260 x 260 mm, pixel size 0.508 x 0.508 mm, slice thickness 5 mm, inter-slice gap 0 mm) were acquired from the anterior superior iliac spine to the knee joint space in two overlapping blocks. Oil filled capsules placed on the lateral side of the thigh allowed alignment of the blocks during analysis. The quadriceps femoris (QF) muscles (vastus lateralis [VL] vastus intermedius [VI], vastus medialis, and rectus femoris) were manually outlined in every third image (i.e. every 1.5 cm) starting from the most proximal image in which the muscle appeared. The volume of each muscle was calculated using cubic spline interpolation (GraphPad Prism 6, GraphPad Software, Inc.). Total QF volume (QUADSvol) was the sum of the individual muscle volumes.

As previously described, the deep aponeurosis of the vastus lateralis muscle was defined as the visible dark black segment between the VL and VI muscles in the thigh MRI images.[32] VL aponeurosis width was defined as the transverse length (cm) of the deep aponeurosis (distinct black segment) between the vastus lateralis and vastus intermedius, traced manually on every third image (i.e. every 1.5 cm), starting in the most distal image where the aponeurosis was visible. Aponeurosis width measures were plotted against the longitudinal aponeurosis length (distance between most proximal and distal image where the aponeurosis was visible [cm]). The surface area of VL aponeurosis was calculated as the area under a spline curve fitted to the aponeurosis width and length plot, and termed VL aponeurosis area (Figure 5).

Immediately after thigh imaging, a lower extremity knee coil was used to acquire axial (time of repetition/time to echo 510/14, image matrix 512 x 512, field of view 160 x 160 mm, pixel size 0.313 x 0.313, slice thickness 2 mm, inter-slice gap 0 mm) and sagittal images (time of repetition/time to echo 480/14, image matrix 512 x 512, field of view 160 x 160 mm, pixel size 0.313 x 0.313, slice thickness 2 mm, inter-slice gap 0 mm) of the knee joint. Contiguous axial images spanned patellar tendon length, which during analysis, were reconstructed to be aligned perpendicular to the line of action of the patellar tendon: straight line from the tendons posterior fibres insertion at the patellar apex to the posterior fibres tibial insertion. Images spanned from 2 cm superior to the patellar apex to 2 cm inferior to the tendon tibial insertion Patellar tendon CSA (mm^2) was measured on each contiguous image along the tendons length (first image where the patellar was no longer visible to the last image before the tibial insertion). Images, viewed in greyscale, were sharpened and the perimeter manually outlined. A spline curve was fitted to the tendon CSA values from each image and the average of the spline equated to mean patellar tendon CSA (patellar tendon mean CSA). The average of the spline CSA's measured over proximal, middle and distal thirds was defined as proximal, mid and distal patellar tendon region CSA. Sagittal plane images were used to determined patellar tendon moment arm, the perpendicular distance from the patellar tendon line of action to the tibio-femoral contact point, which was the midpoint of the distance between the tibio-femoral contact points of the medial and lateral femoral condyles.

Ramp Contractions for Determination of Tissue Stiffness

Tissue stiffness was derived from synchronous recordings of torque and tissue elongation (see below, corrected for passive tissue displacement via video recording of knee joint changes) during isometric knee extension ramp contractions. Participants completed two sub-maximal (~75% MVT) practice ramp contractions prior to five maximal attempts with 90 s rest between contractions. Prior to each ramp contraction participants were shown a target torque-time trace on a computer monitor that increased at a constant gradient (50 Nm.s^{-1} loading rate) from zero up to MVT. They were instructed to match the target trace as closely as possible for as long as possible (i.e. up to MVT), and real-time torque was displayed over the target torque-time trace for feedback. The preceding knee extensor MVCs and sub-maximal contractions were considered sufficient to elicit tissue preconditioning. The three most suitable ramp contractions, according to highest peak torque, the closeness to the target loading rate and ultrasound image clarity, were analysed and measurements averaged across these three contractions.

Measurement of Tissue Elongation

Video images from two ultrasound machines and one video camera were captured to obtain tissue and knee joint displacements during ramp contractions. An ultrasound probe (7.5 MHz linear array transducer, B-mode, scanning width 60mm and depth 50 mm; Toshiba Power Vision 6000, SSA-370A: Otawara-Shi, Japan) was fitted into a custom made high-density foam cast that was strapped to the lateral aspect of the thigh with the mid-point of the probe positioned at ~50 % thigh length. The probe was aligned so the fascicles inserting into the vastus lateralis (VL) muscle deep aponeurosis could be visualized at rest and during contraction. An echo-absorptive marker (multiple layers of transpore medical tape) was placed beneath the ultrasound probe to provide a reference for any probe movement over the skin. Another ultrasound probe (5-10 MHz linear array transducer, B-mode, scanning width 92 mm and depth 65 mm, EUP-L53L; Hitachi EUB-8500) was fitted into a custom made high-density foam cast that was held firmly over the anterior aspect of the knee with the probe aligned longitudinal to the patellar tendon such that the patellar apex and insertion of the posterior tendon fibres at the tibia could be visualized at rest and throughout the contraction. The ultrasound machines were interfaced with the computer collecting torque data in Spike 2 and the video feeds were recorded synchronously with torque using Spike 2 video capture at 25 Hz. During off-line analysis tissue elongation was tracked frame-by-frame using public-domain (www.cabrillo.edu/~dbrown/tracker) semi-automatic video analysis software: Tracker, version 4.86. The distance measured over the surface of the skin between the echo-absorptive marker on the VL and the tibial tuberosity defined resting tendon-aponeurosis complex length. VL fascicle deep aponeurosis cross point displacement relative to the skin marker provided a measure of tendon-aponeurosis elongation. Patellar tendon elongation was determined by the longitudinal displacement of the patella apex and the tendon tibial insertion. The distal insertion of the patellar tendon was not monitored for the purpose of estimating overall tendon-aponeurosis displacement. To enable correction of tissue displacement due to joint angle changes during ramp contractions individual ratios of tissue displacement relative to joint angular displacement (mm/°) were obtained from passive movements (i.e. plotting the tissue displacement-knee joint angle relationship). This ratio was used to determine tissue displacement resulting from knee angle change during ramp contractions, which was subsequently subtracted from total measured displacement. Corrections were only applied to aponeurosis displacement. Tendon elongation under passive conditions was deemed negligible. Passive movements were conducted prior to the ramp contractions. Participants were instructed to completely relax as their knee was moved through 90 to 130°. During passive movements and ramp contractions, knee joint angle (angle between visible markers placed on the greater trochanter, lateral knee joint space and lateral malleolus) was derived from sagittal plane video recorded using a camera mounted on a tripod positioned (1.5 m) perpendicular to the strength-testing chair. The video camera was interfaced with a computer and recorded using spike 2 video capture at 25 Hz (simultaneously with force, EMG, and ultrasound images during the ramp contractions) and analysed via Tracker software.

Calculation of Tendon Force

Patellar tendon force was calculated by dividing external absolute knee extensor torque by the patellar tendon moment arm length. Direct measures of moment arm where acquired at rest from MRI images as indicated above (*MRI measurement*). Due to constraints in the size of the knee coil, sagittal images were acquired in an extended knee position (~163°). Moment arm length for any specific knee angle measured at rest or during ramp contraction was estimated from previously published data fitted with a quadratic function[64] scaled to each participant's measured moment arm length at 163°. Absolute internal knee extensor torque was given by summing net knee extension torque and the estimated knee flexor co-contraction torque. Antagonist knee flexor torque was estimated by expressing the average knee flexor EMG amplitude (RMS 50 ms moving window) during ramp contractions relative to the knee flexor EMG_{MAX} and multiplying by the knee flexor MVT (assuming a linear relationship between EMG amplitude and torque). During analysis, torque and EMG amplitude were down-sampled to 25 Hz to match the ultrasound video frequency.

Calculation of Tissue Stiffness and Tendon Young's Modulus

For each of the three best ramp contractions analysed, tendon-aponeurosis (corrected for passive tissue displacement) and patellar tendon elongation was plotted against total tendon force (corrected for antagonist force). Force-elongation plots were fitted with a second-order polynomial. Tendon-aponeurosis and patellar tendon stiffness was calculated as the gradient (Δ tendon force [N]/Δ elongation [mm]; $N.mm^{-1}$) of the respective force-strain curve over 80-100% (3360-4200N) of an absolute tendon force (4200N) that corresponded to the lowest common force level attained by all participants during ramp contractions. Tendon stress was obtained by dividing tendon force by mean tendon CSA. Tendon strain was the percentage tendon displacement relative to the resting tendon length. Resting PT length was defined as the distance between the patella apex and tibial insertion as measured prior to the ramp contractions. A patellar tendon stress-strain curve was plotted and patellar tendon Young's modulus (GPa) calculated as the slope (Δ tendon stress [MPa]/Δ tendon strain [%]) of the stress-strain curve derived over 80-100% of an absolute common stress (40 MPa). The stiffness and Young's modulus measures derived from each of the three ramp contractions analysed was averaged to give each individuals representative values.

Reproducibility and Statistical Analysis

The reproducibility of tendinous tissue measurements over the duplicate test sessions was calculated for the whole cohort (test 1 vs test 2) as within participant co-efficient of variation (CVw, %; [SD/mean]*100): elongation [0-4200 N] of the patellar tendon (7.9%) and tendon-aponeurosis complex (9.6%); stiffness [3360-4200 N] of the patellar tendon (9.9%) and tendon-aponeurosis complex (8.6%); patellar tendon strain (8.0%) and Young's modulus (9.0%). Patellar tendon CSA measurements were highly reproducible, as indicated by the co-efficient of variation (CV_W) of repeat measurements 12 weeks apart for a sub-sample of the untrained control group (n=14): mean (2.7%), proximal (3.0%), mid (3.1%) and distal (3.5%).

Muscle strength and tissue mechancial/material properties measured during the duplicate laboratory sessions were averaged to produce criterion values for statistical analysis. An a priori significance level of $P<0.05$ was set for all statistical tests which were performed using SPSS Version 20.0 (IBM Corp., Armonk, NY). Descriptive data are presented as mean ± standard deviation (SD) and percentage differences in the group means are given in the text. The influence of group (UC, STT, LTT) on all muscle and tendinous tissue variables was examined by univariate ANOVA. Main group effects were followed by least significant difference (LSD) post-hoc paired comparisons to delineate between group differences; Holm-Bonferroni corrections were applied to LSD P-values, and between group Hedges g effect size (ES) was calculated.[65] Effect size magnitude was classified as <0.2= "trivial"; 0.2-0.6 = "small"; >0-6-1.2 = "moderate"; >1.2-2.0 = "large"; >2.0 = "very large".[66]

Acknowledgements

None to declare

Competing Interests

None to declare

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Physiological Relevance

The potential capacity for tendinous tissue to adapt to long-term functional overload via heavy resistance training is unclear. Our cross-sectional data show that short-term (12 weeks) and long-term (~ 4 years) resistance trained males had similar patellar tendon mechanical and material (Young's modulus) stiffness, with both resistance-trained groups having stiffer tendons that untrained controls. In contrary, neither resistance-trained group had larger tendon size. Furthermore, no differences in tendon-aponeurosis complex stiffness were observed between groups, despite substantially greater muscle strength and size in the long-term resistance trained group, which was also accompanied by a larger muscle-aponeurosis size.

Table 1. Descriptive characteristics of the participants.

	UC	STT	LTT	ANOVA (P)
Sample size, n =	39	15	16	
Age, years	25 ± 2	25 ± 2	22 ± 2	<0.001
Height, cm	176 ± 6	175 ± 8	183 ± 6	0.001
Body mass, kg	72 ± 9	70 ± 9	90 ± 10	<0.001
Tendon-aponeurosis complex length, mm	336 ± 16	328 ± 17	358 ± 18	<0.001
Patellar tendon length, mm	47.7 ± 5.5	45.1 ± 5.5	52.1 ± 5.9	0.005
Patellar tendon moment arm, mm	43.8 ± 2.7	44.8 ± 3.1	45.8 ± 2.5	0.054

Data are mean ± SD.

Table 2. Regional patellar tendon cross-sectional area (mm^2).

Region	UC (n=39)	STT (n=15)	LTT (n=16)	ANOVA (P)
Proximal	93.0 ± 9.9	92.0 ± 13.5	98.4 ± 13.1	0.216
Mid	104.4 ± 12.6	97.0 ± 14.3	104.9 ± 13.3	0.146
Distal	110.4 ± 17.9	101.6 ± 14.3	112.1 ± 15.7	0.141

Data are mean ± SD.

Table 3. Summary of group differences in strength (maximal voluntary torque, MVT), muscle tendon unit size and tissue (tendon-aponeurosis complex and patellar tendon [PT]) stiffness between long-term resistance trained (LTT), short-term resistance trained (STT) and untrained control (UC) groups.

Function	
MVT, Nm	LTT > STT > UC
Muscle-Tendon Unit size	
QUADSvol, cm^3	LTT > STT & UC
VL Aponeurosis Area, cm^2	LTT > STT & UC
PT CSA, mm^2	-
Indices of Tissue Stiffness	
PT elongation (0-4200 N), mm	LTT < STT & UC
PT stiffness (3360-4200 N), $N.mm^{-1}$	LTT & STT > UC
PT strain (0-40 MPa), %	LTT < STT & UC
PT Young's modulus (32-40 MPa), GPa	LTT & STT > UC
Tendon-aponeurosis elongation (0-4200 N), mm	-
Tendon-aponeurosis stiffness (3360-4200 N), $N.mm^{-1}$	-

Significant group differences groups: greater (>) or less (<) than. No difference (-). PT CSA: both mean and regional CSA measures.

Figure Legends

Figure 1. Group comparisons: Isometric knee extension maximal voluntary torque (a), Quadriceps femoris muscle volume (QUADSvol, b), Vastus Lateralis (VL) aponeurosis area (c) and Patellar tendon mean cross-sectional area (CSA, d) for untrained control (UC, n = 39), short-term resistance trained (STT, n = 15) and long-term resistance trained (LTT, n = 16) groups. Data are mean ± SD. Bold numbers are between groups hedges g effect size. Post-hoc tests: Least significant difference Holm-Bonferroni corrected P-values. *P<0.05, †P<0.01, ‡P<0.001.

Figure 2. (a) Relationships between patellar tendon force (N) and elongation (mm) in the untrained control (UC, n = 37), short-term resistance trained (STT, n = 15) and long-term resistance trained (LTT, n = 15) groups. Curves show the group mean relationship. Data points correspond to within group average values for the elongation at 10% intervals of group mean maximal voluntary tendon force, plotted up to 80% (highest common level achieved during ramp contractions). Error bars indicate the within-group standard deviation for force (y-axis bar) and elongation (x-axis bar). **(b) And (c)** Group comparisons of the patellar tendon elongation at the common force level of 4200 N, and patellar tendon stiffness (gradient of curves in **a** over 80-100% of the highest common force level [3360-4200 N]). Bars are mean ± SD. Bold numbers are the between groups hedges g effect size. Post-hoc tests: Least significant difference Holm-Bonferroni corrected P-values. *P<0.05, ‡P<0.001.

Figure 3. (a) Relationships between patellar tendon stress (MPa [N.mm^2]) and strain (%) in the untrained control (UC, n = 37), short-term resistance trained (STT, n = 15) and long-term resistance trained (LTT, n = 15) groups. Curves show the group mean relationship. Data points correspond to within group average values for the strain at 10% intervals of group

mean maximal voluntary tendon stress, plotted up to 80% (highest common level achieved during ramp contractions). Error bars indicate the within-group standard deviation for stress (y-axis bar) and strain (x-axis bar). **(b) And (c)** Group comparisons of the PT strain at the common stress level of 40 MPa, and PT Young's modulus (gradient of curves in **a** over 80-100% common stress level [32-40 MPa]). Bars are mean ± SD. Bold numbers are the between groups hedges g effect size. Post-hoc tests: Least significant difference Holm-Bonferroni corrected P-values. †P<0.01.

Figure 4. (a) Relationships between patellar tendon force (N) and tendon-aponeurosis complex elongation (mm) in the untrained control (UC, n = 37), short-term resistance trained (STT, n = 14) and long-term resistance trained (LTT, n = 16) groups. Curves show the group mean relationship. Data points correspond to within group average values for the strain at 10% intervals of group mean maximal voluntary tendon force, plotted up to 80% (highest common level achieved during ramp contractions). Error bars indicate the within-group standard deviation for force (y-axis bar) and strain (x-axis bar). **(b) And (c)** Group comparisons of the tendon-aponeurosis complex elongation at the common force level of 4200 N, and tendon-aponeurosis complex stiffness (gradient of curves in **a** over 80-100% common force level [3360-4200 N]). Bars are mean ± SD.

Figure 5. Measurement of the surface area of the vastus lateralis deep aponeurosis. On axial magnetic resonance images at 1.5 cm intervals along the longitudinal aponeurosis length: distance from the most distal image to the most proximal where the aponeurosis was visible (a), the transverse length of the distinct visible black segment between the vastus lateralis (VL) and vastus intermedius (VI) muscles was traced manually and defined as aponeurosis width (b). The aponeurosis width measures on each image were plotted against aponeurosis

length and the area under a cubic spline curve fitted through the data points defined the VL aponeurosis area (c).

Figure 1.

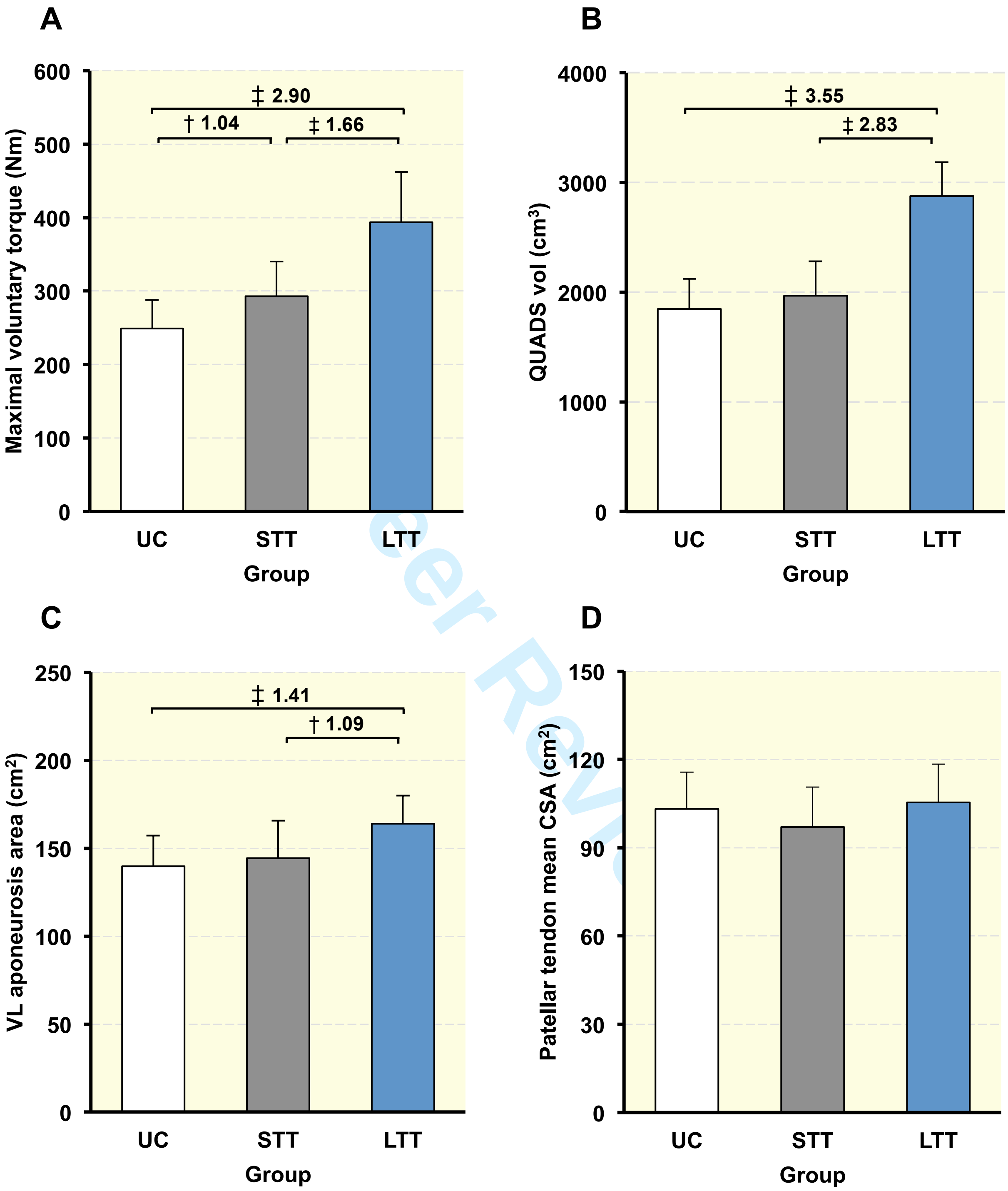

A
Maximal voluntary torque (Nm)
600
500
400
300
200
100
0
‡ 2.90
† 1.04
‡ 1.66
UC
STT
LTT
Group
B
QUADS vol (cm³)
4000
3000
2000
1000
0
‡ 3.55
‡ 2.83
UC
STT
LTT
Group
C
VL aponeurosis area (cm²)
250
200
150
100
50
0
‡ 1.41
† 1.09
UC
STT
LTT
Group
D
Patellar tendon mean CSA (cm²)
150
120
90
60
30
0
UC
STT
LTT
Group

Figure 2.

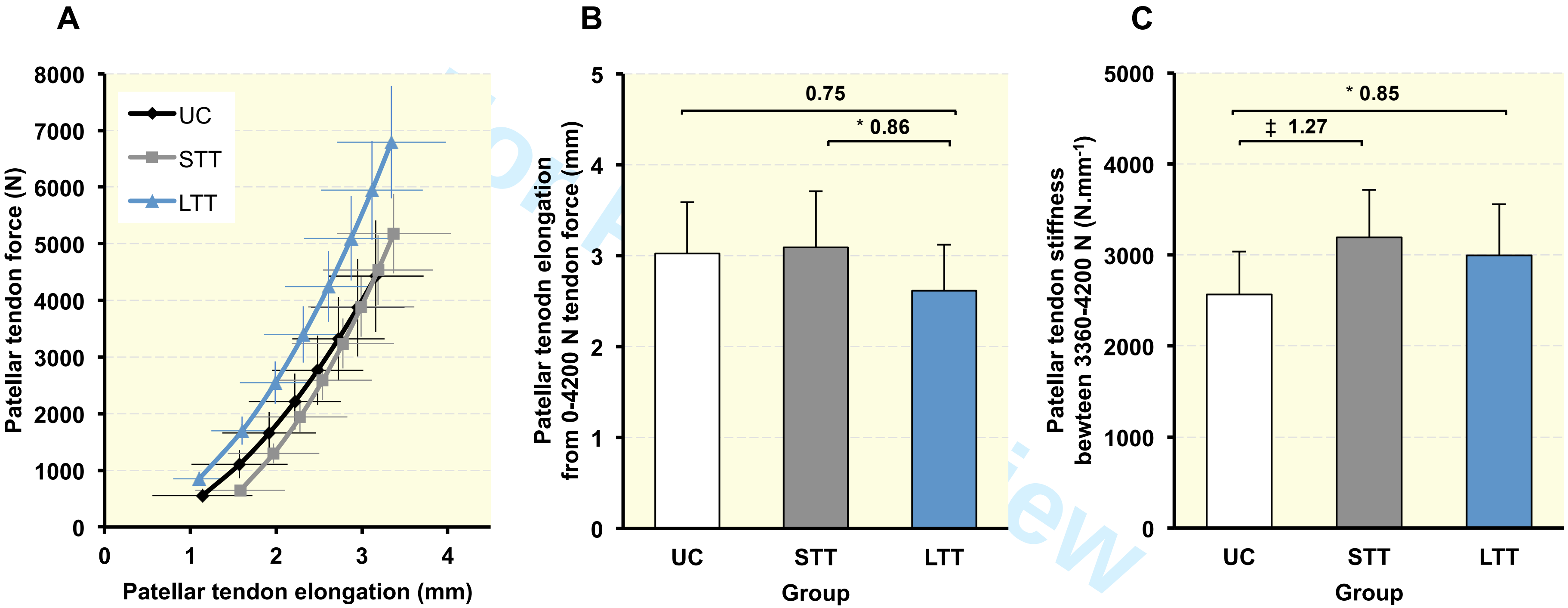

A
8000
7000
6000
5000
4000
3000
2000
1000
0
UC
STT
LTT
Patellar tendon force (N)
0
1
2
3
4
Patellar tendon elongation (mm)
B
5
4
3
2
1
0
0.75
* 0.86
Patellar tenodn elongation
from 0-4200 N tendon force (mm)
UC
STT
LTT
Group
C
5000
4000
3000
2000
1000
0
* 0.85
‡ 1.27
Patellar tendon stiffness
bewteen 3360-4200 N (N.mm^{-1})
UC
STT
LTT
Group

Figure 3.

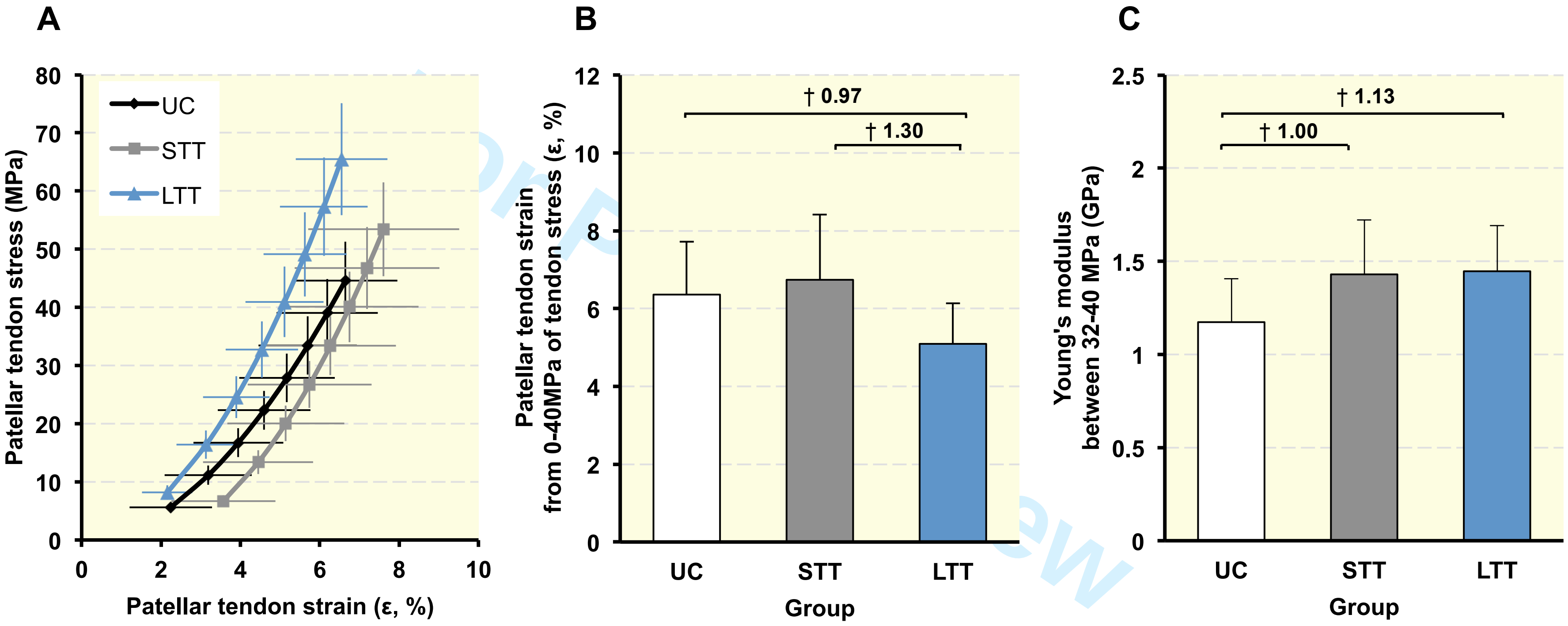

A
B
C
80
70
60
50
40
30
20
10
0
Patellar tendon stress (MPa)
0
2
4
6
8
10
Patellar tendon strain (ε, %)
UC
STT
LTT
12
10
8
6
4
2
0
Patellar tendon strain from 0-40MPa of tendon stress (ε, %)
† 0.97
† 1.30
UC
STT
LTT
Group
2.5
2
1.5
1
0.5
0
Young's modulus between 32-40 MPa (GPa)
† 1.13
† 1.00
UC
STT
LTT
Group

Figure 4.

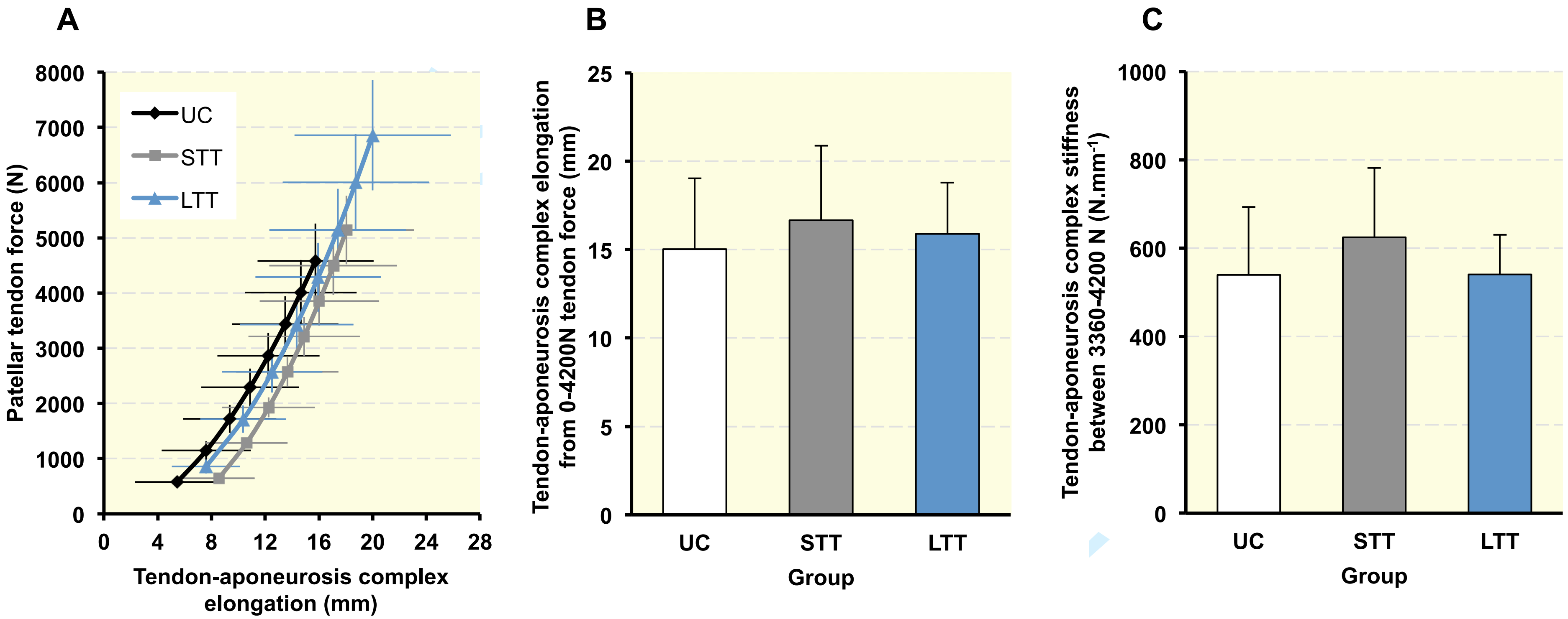
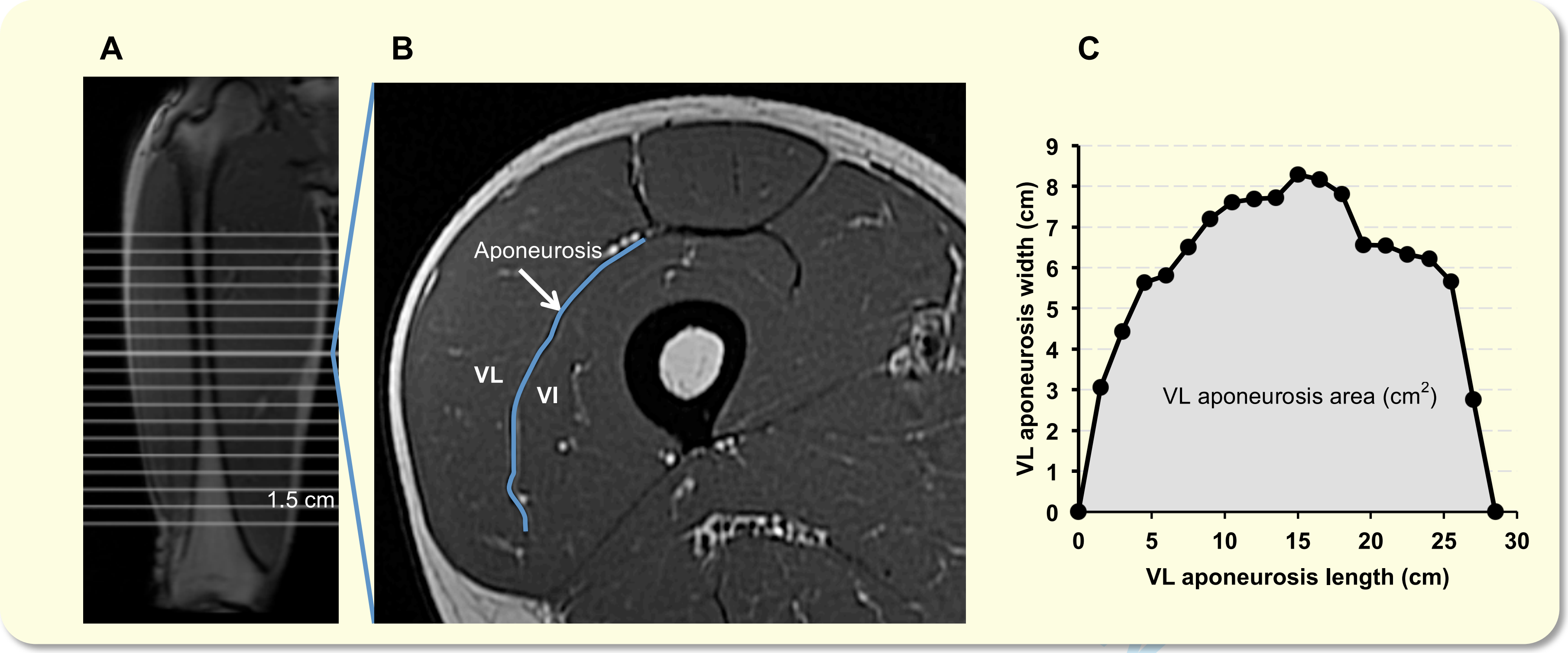

A
Patellar tendon force (N)
8000
7000
6000
5000
4000
3000
2000
1000
0
0
4
8
12
16
20
24
28
Tendon-aponeurosis complex elongation (mm)
UC
STT
LTT
B
Tendon-aponeurosis complex elongation from 0-4200N tendon force (mm)
25
20
15
10
5
0
UC
STT
LTT
Group
C
Tendon-aponeurosis complex stiffness between 3360-4200 N (N.mm-1)
1000
800
600
400
200
0
UC
STT
LTT
Group

Figure 5.


A
B
C
1.5 cm
Aponeurosis
VL
VI
VL aponeurosis width (cm)
0
1
2
3
4
5
6
7
8
9
VL aponeurosis area (cm²)
0
5
10
15
20
25
30
VL aponeurosis length (cm)