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Original article

Patellar tendon adaptations to downhill running training and their relationships with changes in mechanical stress and loading history

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Running title: Tendon adaptations to downhill running

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ABSTRACT

It is unclear if human tendon adapts to *moderate-intensity*, *high-volume* chronic eccentric exercise, e.g. downhill running (DR) training. This study aimed to investigate the time course of patellar tendon (PT) adaptation to short-term DR training, and to determine if changes in PT properties were related to changes in mechanical stress and/or loading history. Twelve untrained, young, healthy adults (five women, seven men) took part in four weeks' DR training, comprising 10 sessions. Running speed was equivalent to 60-65% $\dot{V}O_{2max}$, and session duration increased gradually (15-30min) throughout training. Isometric knee-extensor maximal voluntary torque (MVT), *vastus lateralis* (VL) muscle physiological cross-sectional area (PCSA) and volume, and PT CSA, stiffness and Young's modulus were assessed at weeks 0, 2 and 4 *via* ultrasound and isokinetic dynamometry. PT stiffness (+6.4±7.4%), Young's modulus (+6.9±8.8%), isometric MVT (+7.5±12.3%), VL volume (+6.6±3.2%), PCSA (+3.8±3.3%), increased after four weeks' DR (p<0.05), with no change in PT CSA. Changes in VL PCSA correlated with changes in PT stiffness (r=0.70; p=0.02) and Young's modulus (r=0.63; p=0.04) from 0-to-4 weeks, while changes in MVT did not correlate with changes in PT stiffness and Young's modulus at any time point (p>0.05). To conclude, four weeks' DR training promoted substantial changes in PT stiffness and Young's modulus that are typically observed after *high-intensity, low-volume* resistance training. These tendon adaptations appeared to be driven primarily by loading history (represented by VL muscle hypertrophy), while increased mechanical stress throughout the training period did not appear to contribute to changes in PT stiffness or Young's modulus.

Keywords: eccentric training; endurance training; strength; tendon stiffness; Young's modulus

INTRODUCTION

Tendon is a metabolically active tissue, displaying remarkable adaptability to increased loading (3). Evidence suggests that chronic exposure to overloading may promote *in vivo* changes in the morphological (e.g. cross-sectional area, CSA), mechanical (e.g. tensile stiffness) and/or material properties (e.g. Young's modulus) of tendon (49). Such adaptations are necessary to ensure the tendon is capable of transmitting greater forces generated by the muscle (as a consequence of the muscle's adaptation to increased loading) to the bone (34). Conventional (i.e. concentric-eccentric) resistance training, as well as flywheel eccentric exercise, have been reported to increase tendon stiffness (17, 35, 42, 44), and tendon adaptations were identified *in vivo* after only a few weeks' resistance training in some of these studies (17, 42). Because force-generating capacity is greater during eccentric *versus* concentric and isometric contractions (23), the former allows the use of heavier loads (43). As such, it has been speculated that high-intensity resistance training involving eccentric contractions might cause the tendon to undergo greater strain, which may represent a stronger stimulus for tendon hypertrophy (21, 22) and changes in mechanical properties *in vivo* (15, 29, 35, 42). However, some *in vivo* human studies have reported increased collagen protein turnover in the days following acute *moderate-intensity*, *high-volume* exercise, such as running or repetitive kicking exercises (11, 31, 38), thus suggesting that high strain is not the only prerequisite to tendon adaptation.

Loading history has been suggested as a major driver of tendon adaptations to chronic exercise. This hypothesis was supported by Seynnes et al. (44), who reported a strong correlation between the increase in patellar tendon stiffness and quadriceps femoris muscle hypertrophy after nine weeks' conventional resistance training in young men. Interestingly, side-to-side habitual loading has been associated with asymmetrical tendon remodelling and mechanical properties (10), highlighting the influence of loading history in human tendon

adaptation. Furthermore, there are numerous cross-sectional studies, reporting larger Achilles tendon (33, 48, 51) and patellar tendon (48, 51) CSA in endurance runners *versus* untrained controls. This suggests that both the Achilles tendon and patellar tendon adapt to endurance running by hypertrophying (4, 30, 33, 50).

To date, however, the exact nature, magnitude, and time course of *in vivo* changes in human tendon mechanical and material adaptations to running training are poorly understood. To the best of our knowledge, only one longitudinal study has investigated the effects of endurance running training on tendon adaptations in previously untrained, healthy individuals (20). While nine months' regular running training induced cardiovascular improvements, Achilles tendon CSA and stiffness remained unchanged. In this context, it appears that chronic level running training does not affect Achilles tendon properties and/or the overloading characteristics were not optimal to stimulate such adaptations. It should be emphasized that gastrocnemius fascicles shorten throughout the entire stance phase during running (39), highlighting the necessity of the long gastrocnemius tendon to elongate during the stance phase. Moreover, the storage and release of elastic energy from the Achilles/gastrocnemius tendon during walking/running is extremely efficient and important for running performance (51). Conversely, the patellar tendon likely plays a different role during running due to its greater CSA and stiffness, and appears to be better suited for an effective transmission of contractile force than the Achilles tendon (39). This could be related to the high forces generated by the quadriceps during running to decelerate and support body mass (12, 41). Interestingly, the external forces exerted on the quadriceps muscle-tendon unit are even greater during downhill running (DR) (41), where higher peak vertical and horizontal braking impact forces (19), which are associated with higher and longer negative work at the knee over the stance phase, may result in greater internal quadriceps force generation during DR (8). Accordingly, DR may elicit greater stress on the patellar tendon, altering its mechanical and material properties.

Concomitantly, DR is characterized by the repetition of submaximal intensity eccentric contractions of the knee extensors, a specific loading history that could represent a unique stimulus for the musculotendinous system (e.g. chronic *moderate-intensity*, *high-volume* eccentric exercise). It has speculated that downhill walking/running could represent a viable overloading strategy in clinical and/or athletic settings to promote adaptations to the muscle-tendon complex in a more ecological context, as it closely mimics daily activities and/or the main activity of athletes (7, 18). This is particularly true for endurance athletes, especially runners, who could benefit from this alternative approach. While it is well-described that resistance training leads to rapid adaptations in the musculotendinous system, less is known about the effects DR training. Nevertheless, short-term periodized DR training (4-5 weeks) has been shown to promote lower limb muscle strengthening and adaptations (e.g. increased muscle size and fascicle length) (6, 46), thus having potentially positive implications for athletic performance and injury risk reduction. If incorporating DR into a runner's training program could also increase patellar tendon stiffness, this could lead to a more effective transfer of force from the knee extensor muscles during each stride, thus improving running economy (1). However, to date, no study has investigated the effects of DR training on patellar tendon adaptations.

Hence, the purpose of the present study was to investigate the time course of human patellar tendon adaptations to four weeks' DR training. We hypothesized that DR training would lead to increased patellar tendon stiffness and Young's modulus, which would be related to knee extensor muscle hypertrophy but not strength gains, thus suggesting that loading history would be the primary stimulus for patellar tendon adaptations to DR training.

METHODS

Experimental Approach to the Problem

A longitudinal study with repeated measures design was used to examine changes in patellar tendon properties following four weeks' *moderate-intensity, high-volume* eccentric training in healthy, recreationally active individuals. DR was specifically chosen as an exercise modality to promote mechanical overloading of the quadriceps femoris muscle-tendon unit. Moreover, this population was chosen to maximise the potential DR training-induced changes in our population over a short period of time. Participants attended the laboratory on 12 separate occasions. During the first visit, participants performed a maximal running test to exhaustion to determine maximal oxygen uptake ($\dot{V}O_{2max}$). The subsequent 11 visits were allocated to DR training sessions and/or testing (visits seven and 12, Table 1). Muscle strength, *vastus lateralis* (VL) muscle morphology and patellar tendon properties were evaluated in the right leg at 0 weeks (i.e., before starting the DR training), 2 weeks (i.e., *before* starting training session #7, 3 to 5 days after completion of the final DR training bout in order to limit exercise-induced muscle damage and fluid shifts into the muscle confounding muscle size and strength measurements) and 4 weeks (i.e., also 3 to 5 days after completion of the final DR training bout for the same reasons as mentioned above). During those testing sessions, the following physiological assessments were performed in this order: VL and patellar tendon morphological properties, knee extensor and flexor isometric maximal voluntary torque (MVT), followed by the patellar tendon mechanical and material properties assessment.

Subjects

Fifteen participants volunteered to take part in the study but three of them withdrew during the study for personal reasons. Participants were healthy, recreationally active individuals (n = 12; five women and seven men; age: 25.1 ± 4.9 years; height: 1.69 ± 0.08 m; mass: 66.7 ± 13.1 kg; BMI: 23.2 ± 3.3). The number of participants exceeded the estimated minimum sample size (n= 11; one-tailed; α: 0.05; Power: 0.95; G*Power software v3.1.9.6, Heinrich-Heine-Universität

Düsseldorf, Düsseldorf, Germany), which was based on the changes in patellar tendon Young's modulus after four weeks' resistance training in young, healthy men (i.e. 1.05 ± 0.29 GPa and 1.43 ± 0.39 GPa at 0 and 4 weeks, respectively) (42). Although the recruitment of a mixed-sex sample could induce some external variability in terms of musculotendinous adaptations to training (43), it nevertheless allows a better understanding of how the patellar tendon adapts to the chronic use of DR in a more ecologically valid and representative sample of a young, healthy population of recreationally active men and women. Participants were free from any medical contraindications and had no history of musculotendinous injuries, or plyometric, eccentric and/or heavy resistance training in the six months prior to the study. They had also never performed any DR-specific training. Further, they were asked to maintain habitual lifestyle habits and physical activity for the duration of the study (*i.e.* <4 hours' moderate to vigorous physical activity per week, which included recreational running). None of the female participants was using any form of hormonal contraception or long-acting reversible contraceptive (e.g. contraceptive implants and intrauterine devices) in the six months prior to the study, or during the study itself. In addition, female participants were asked to provide information on the typical length of their menstrual cycle and the number of days since the start of their last menstrual cycle (i.e. first day of menstruation). The self-reported typical menstrual cycle was used to estimate the day of peak luteinizing hormone concentration using the regression equation of McIntosh et al. (37), rounded to the nearest whole day. This allowed the pre- and post-training assessments to be determined and scheduled as close as possible to the start of the follicular phase (i.e. ± 48 h to the first day of menstruation), thereby reducing any potential effect of oestrogen fluctuation on tendon properties (9). Subjects were informed of the experimental procedures and gave their written informed consent before the study commenced. All procedures were approved by Liverpool John Moores University Research Ethics

Committee (19/SPS/024) and conformed to the standards regarding the use of human participants in research, as outlined in the Sixth Declaration of Helsinki.

Procedures

After performing the maximal running test to exhaustion to determine $\dot{V}O_{2max}$ and having had 20 minutes of rest (first visit), subjects were familiarized with DR at three different slopes (-5%, -10% and -15%; i.e., DR_5, DR_{10} and DR_{15}, respectively) at grade-related speeds associated with 60-65% $\dot{V}O_{2max}$ for 10-15 min. Further, this first session enabled the participants to be familiarised with all other experimental procedures. The subsequent 11 visits were allocated to DR training sessions and/or testing (visits seven and 12) sessions (Table 1). The DR training was conducted on a motorized treadmill (HP Cosmos, Nussdorf, Germany) for 10 training sessions over 4 consecutive weeks, with one-to-two days' rest allocated between training sessions. Each participant was required to keep the same training schedule for the duration of the study, either in the morning or afternoon (± 1.5 h). Each DR training session comprised consecutive treadmill running at DR_5, DR_{10} and DR_{15}, and at a speed associated with a metabolic intensity of 60-65% $\dot{V}O_{2max}$ at each grade (Table 1). Grade-related speeds were 8.5 ± 0.9 $km \cdot h^{-1}$, 10.2 ± 1.6 $km \cdot h^{-1}$, 11.7 ± 1.9 $km \cdot h^{-1}$ and 13.0 ± 1.9 $km \cdot h^{-1}$ for the level grade, DR_5, DR_{10} and DR_{15}, respectively. The training volume was gradually increased by enhancing total running time and/or running time at steeper slopes in training sessions to promote significant stress on the patellar tendon. Before each DR training session, participants carried out a warm-up, comprising 7 min level running and 3 min DR_{10} at a speed associated with a metabolic intensity of 60–65% $\dot{V}O_{2max}$. Laboratory conditions remained stable throughout the sessions (temperature: 23.4 ± 1.0°C; relative humidity: 41.7 ± 7.4%).

Please insert Table 1 near here.

Muscle-Tendon Morphological Properties

VL and patellar tendon morphological properties were assessed using a real-time B-mode ultrasound system (Philips EPIQ Elite, Amsterdam, The Netherlands) with a 5-18 MHz 40 mm wide linear array probe. For the VL morphological assessment, the participant was resting supine on an examination bed with the knee fully extended and the ankle fixed in the neutral position. For the patellar tendon, measurements were conducted with the participant resting on an isokinetic dynamometer and the right knee joint set at 90° knee flexion (full extension = 0°) and the hip joint set at 85° (supine = 180°). Regions of interest were identified through ultrasonography, then marked on the skin using a permanent marker pen. Water-soluble gel was generously applied to the transducer to aid acoustic coupling and to minimise the pressure applied to the skin with the transducer. For each measurement location on each participant, at least three images were taken, and the mean of the best three (visually evaluated) was used for subsequent analysis. All ultrasound images were analysed using ImageJ software (National Institutes of Health, Bethesda, MA, USA). Patellar tendon cross-sectional area (CSA) was measured with the transducer positioned transversally to the axis of the tendon and perpendicular to the skin surface. Three different locations were investigated: 25% (proximal), 50% and 75% tendon resting length ($CSA_{25\%}$, $CSA_{50\%}$ and $CSA_{75\%}$, Fig. 1), which was defined as the distance from the patella apex to the tibial tuberosity.

Please insert Fig.1 near here.

Methods regarding the VL muscle morphological and architectural assessments have been described in detail previously (6). Briefly, VL muscle anatomical CSA was measured using the extended-field-of-view function of the ultrasound system (Philips EPIQ Elite), with the

transducer moving along the transverse plane of the VL at three different locations: 25% (proximal), 50% and 75% muscle length (Fig. 1). Muscle volume was then calculated using the truncated cone method. Fascicle length was determined using extended-field-of-view ultrasound images from 20% to 80% of VL muscle length (along the mid-sagittal plane, perpendicular to the skin and carefully aligned to the direction of the fascicles) to identify entire fascicular paths. VL muscle physiological CSA (PCSA) was calculated by diving VL muscle volume by fascicle length.

Isometric KE and KF MVT

Isometric maximal voluntary torque (MVT) was assessed using an isokinetic dynamometer (Humac Norm, CSMI, Massachusetts, USA), with the hip joint set at 85° (supine = 180°) and the participant's chest, waist and thigh secured to the chair with inextensible straps. The warm-up comprised 10 concentric repetitions ($30°\cdot s^{-1}$) performed with increasing intensity, i.e. ~10% to ~80% of perceived maximum effort, followed by two repetitions at ~80% isometric MVT. Thereafter, participants performed two 3-s knee extensors MVT with the knee joint set at 90° knee flexion, interspersed by 60-s passive rest. This was followed by two three-second knee flexors MVT, with the same knee joint and rest protocol. Real-time visual and verbal feedback regarding torque production, as well as verbal encouragement, was provided throughout the test. If the second knee extensors or flexors MVT was >5% higher than the first, a further attempt was made until the variability between the highest MVT, and the next highest was <5%. For all contraction modalities, the highest MVT was used for subsequent analysis. Torque data were sampled at 2 kHz using data acquisition software (AcqKnowledge, Biopac Systems Inc., Goleta, CA, USA) and low-pass filtered at 20 Hz.

Patellar Tendon Elongation During a Ramped MVC (RMVC)

Patellar tendon mechanical and material properties were assessed by measuring the tendon elongation over ramp maximal voluntary contraction (RMVC) (44). A second real-time B-mode ultrasonography system (Mylab70, Esaote, Genoa, Italy) with a 10−15 MHz *100 mm* wide linear array probe was used to measure tendon elongation. These specific methodological considerations (e.g. wider probe) enabled us to track displacement of both the patella apex and tibial tuberosity simultaneously throughout the RMVC. All RMVCs were performed on the isokinetic dynamometer and with the chair set up as for the MVT. Prior to performing RMVCs, a 2-mm-wide strip of surgical tape (3M, Neuss, Germany) was placed on the skin transversely over the long axis of the tendon at ~50% tendon length, which acted as an echo-absorbent reference point, enabling the detection of any movement of the transducer during the RMVC (in which case, the RMVC data were discarded, and the procedure was repeated). Warm-up and MVT tests were performed prior to performing RMVCS (~ 90s) to ensure tendon preconditioning. The transducer was placed on the skin over the tendon in the mid-sagittal plane and the RMVC contraction phase lasted 6 s, with 2 min rest between each contraction. Loading rates ($N \cdot m \cdot s^{-1}$) were measured off-line and were, therefore, dependent on the participant's maximal force production capacity. To ensure that all RMVCs were performed at constant loading rates, visual feedback of force production and time were displayed on a screen in front of the participants. Trials were discarded if the torque trace deviated from the required linear pattern upon visual inspection. In this particular case, participants performed a third RMVC. The torque signal was interfaced with an analogue-to-digital converter (MP150 Biopac Systems Inc., Santa Barbara, USA), sampled at 2 kHz with a PC using data acquisition software (AcqKnowledge v.5.1, Biopac Systems Inc.), low-pass filtered (20 Hz edge frequency), then resampled offline at 25 Hz. Further, torque data were synchronized with ultrasound video sequences (25 Hz) via the administration of a square wave pulse, which was visible simultaneously on the AcqKnowledge software and the ultrasound movie file via the ECG

signal. Due to a technical issue encountered with one participant at week two, the sample size regarding muscle morphology and tendon CSA assessments was reduced to *n*=11. In another participant, the tendon insertions were not clearly identifiable during RMVCs, which meant that sample size regarding tendon stiffness was limited to *n*=11 at all time points. Thus, since the calculation of Young's modulus requires tendon CSA, the sample size for this measurement at week two was *n*=10.

Antagonist Muscle Co-activation

The electromyographic (EMG) activity of knee flexors was assessed during MVT and RMVCs to measure antagonist muscle co-activation. Surface EMG activity was recorded from the right biceps femoris long head (BFlh) using Ag-AgCl bipolar electrodes (Blue Sensor N-00-S, Ambu, Denmark). Following preparation of the skin (shaving, lightly abrading and cleansing with 70% ethanol), two electrodes were attached (20 mm apart) on the skin at a location corresponding to the distal third of the BFlh muscle length along the mid-sagittal plane (http://www.seniam.org/). All electrode locations (with regard to distances from anatomical landmarks) were measured and recorded for relocation during subsequent tests. Surface EMG signals were amplified [within a bandwidth frequency ranging from 10-500 Hz (common-mode rejection ratio >85 dB, gain = 1000)] using the same analogue-to-digital converter (MP150 Biopac Systems Inc.) as the torque signal, prior to being band-pass filtered in both directions between 10 and 500 Hz then resampled at 25 Hz. The root mean square (RMS) of the EMG signal was used to assess BFlh activation during RMVC. Assuming a linear relationship between EMG activity and torque output (25), the torque generated by the antagonists during knee extension RMVC was estimated by dividing the BFlh EMG during knee extensor RMVC by the BFlh maximal EMG RMS (assessed 300 ms around peak torque during knee flexion MVT) and multiplying this ratio by the knee flexor MVC torque. This torque was then added

to the knee extensor torque value to provide the net knee extensor RMVC torque. Tendon force was thereafter calculated by dividing RMVC torque by the patellar tendon moment arm at 90° knee flexion [assumed to be 0.048 m based on magnetic resonance imaging measurements in young, healthy individuals (14)].

Analysis of Tendon Data

Patellar tendon resting length was defined as the total distance between the patella apex and tibial tuberosity and was measured offline using ImageJ (v. 1.47, National Institute of Health, MD, USA). Patellar tendon elongation during RMVCs was assessed offline using semi-automatic video analysis software (Tracker v. 6.0.10, physlets.org/tracker). Individual force × elongation curves were fitted with a second-order polynomial ($R^2 \geq 0.90$, Fig. 1). Tendon stiffness (Δforce/Δtendon length) and Young's modulus (Δstress/Δstrain) were calculated over the highest 20% common force interval from each participant's weakest (baseline) RMVC. Patellar tendon elongation was calculated as the change in tendon length at the force corresponding to 100% each participant's weakest RMVC from resting length. Tendon strain was calculated as the change in tendon length at each participant's weakest RMVC force relative to the original tendon length ($\Delta L/L_0$), and expressed as a percentage. Tendon stress was calculated by dividing the force corresponding to each participant's baseline RMVC by tendon CSA_{mean}.

Test-Retest Reproducibility

Test-retest reproducibility for assessments of patellar tendon properties was investigated in eleven healthy young, recreational active individuals (eight men and three women; age: 25.5 ± 2.5 years; height: 1.70 ± 0.08 m; mass: 71.5 ± 14.7 kg; BMI: 24.5 ± 3.6). Of these individuals, seven completed the test-retest measurements of patellar tendon mechanical properties, seven

completed the test-retest measurements of patellar tendon morphological properties, and four completed the test-retest measurements of patellar tendon materials properties. Eight of this group also completed the DR training study. Measurements were performed by the same investigator. Inter-day reproducibility (interspersed by 14 to 21 days) was expressed as typical error (TE), coefficient of variation (CV) and intraclass correlation coefficient (ICC, model: two-way mixed; type: absolute agreement) with 95% confidence intervals (CIs).

Statistical analyses

Data for all variables are expressed as mean ± standard deviation, and statistical analyses were conducted on absolute data. All data were tested for normality using the Shapiro-Wilks normality test and sphericity was assumed. A one-way ANOVA was used to determine the main effect of *training* only. A two-factor within-subjects ANOVA was also used to determine the main effects of *training* × *location* for tendon CSAs. Mixed-effects models were used, as one data value was missing for one participant at week 2. When significant main effects for one-way ANOVAs occurred, Fisher's LSD post-hoc pairwise comparison tests were used and Cohen's d effect sizes were reported for significant changes. Relationships between independent variables (i.e. percentage changes in MVT and VL PCSA) and dependent variables (i.e. percentage changes in tendon stiffness and Young's modulus) were tested with Pearson's product-moment correlations. Pearson's correlation coefficient (r), the corresponding 95% CIs and the *p*-value were reported for each analysis. The significance level for all analyses was set at $p<0.05$. All statistical analyses were performed using GraphPad Prism software (version 8.0; GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Patellar Tendon Morphological, Mechanical, and Material Properties

For all variables, test-retest reproducibility showed CVs were low, and ICCs were high (≤ 0.90) with narrow CIs (Table 2). All ANOVA and descriptive results are presented in Table 3. A main effect of training was observed for tendon stiffness and Young's modulus only (Table 3). Post-hoc comparisons revealed that tendon stiffness (+6.4 ± 7.4%; p=0.02; Cohen's d=0.23) and Young's modulus (+6.9 ± 8.8%; p=0.02; Cohen's d=0.26) increased at 4 weeks but not at 2 weeks (both p>0.05). No change was observed regarding tendon CSA (neither mean nor at each location along its length) or resting length over the training period (both p>0.05).

Please insert Table 2 near here.

Muscle Strength and Morphological Muscle Changes

A significant main effect of training was observed for MVT but not RMVC tendon force corrected for antagonist muscle co-activation (Table 3). Post-hoc analyses revealed that knee extensor MVT increased by 7.5 ± 12.3% from baseline (p=0.032; Cohen's d=0.28) at four weeks. VL muscle morphological changes have been reported previously (6) and only percentage changes were used in this study for bivariate correlations with percentage changes in tendon properties. Briefly, increases in VL muscle morphology were observed at four weeks, i.e. PCSA (+3.8 ± 3.3%; p<0.001; Cohen's d=0.17) and volume (+6.6 ± 3.2%; p<0.001; Cohen's d=0.28). Our test-retest reproducibility for VL muscle architecture and morphology measurements has been previously reported (6).

Please insert Table 3 near here.

Relationships Between the Changes in Patellar Tendon Mechanical and Material Properties and Changes in Strength and Muscle Morphology

All significant bivariate correlations are presented in Fig.2. Changes in knee extensor MVT did not correlate with changes in patellar tendon stiffness or Young's modulus at any time point (all $p<0.05$), while significant correlations were observed between percentage changes in VL PCSA with changes in patellar tendon stiffness (r=0.70; 95% CIs: 0.18 to 0.92; $p=0.02$) and Young's modulus (r=0.63; 95% CIs: 0.05 to 0.89; $p=0.04$) from 0-to-4 weeks only.

Please insert Fig.2 near here.

DISCUSSION

The purpose of the present study was to investigate the effect of four weeks' *moderate-intensity*, *high-volume* eccentric training on patellar tendon morphological, mechanical, and material properties. Downhill running (DR) was specifically chosen as an exercise modality to promote mechanical overloading of the *quadriceps femoris* muscle-tendon unit (19, 41). After the four-week DR training period, increases in patellar tendon stiffness (+6.4%) and Young's modulus (+6.9%) but not tendon hypertrophy were observed. Increased stiffness and Young's modulus were accompanied by increases in knee extensors MVT (+7.5%) and *vastus lateralis* (VL) muscle PCSA (+3.8%). Changes in patellar tendon stiffness and Young's modulus were not related to increased mechanical stress at any time point throughout training. However, the specific DR training-induced loading history (represented by gains in VL PCSA) seemed to contribute to changes in tendon mechanical and material properties from 0-to-4 weeks. Taken together, these results demonstrate that four weeks' DR training can promote rapid changes in patellar tendon properties in healthy, previously untrained young adults, which seem to be explained predominantly by loading history rather than increased mechanical stress *per se*.

It is well documented that the human patellar tendon adapts to conventional resistance training by increasing its stiffness and modulus and, depending on the measurement location,

its CSA (27, 30, 44). However, prior to this study, it was not known whether the human patellar tendon adapted to chronic running exercise, let alone to DR training. Downhill walking/running has been speculated to be a useful overloading strategy in clinical and/or athletic settings to promote adaptations to the muscle-tendon complex in a more ecological context, i.e. similar to daily activities and/or their main activity (6, 46). We have recently reported that four weeks' DR training induced substantial increases in KE strength and VL muscle size and architecture (6). In the present study, increases in patellar tendon stiffness and Young's modulus were observed after the same short period of DR training. While the time-course of tendon mechanical adaptations is still poorly understood (51), this is not the first study to detect rapid *in vivo* tendon adaptations in response to short-term overloading (17, 42). However, these latter studies reported greater increases in tendon stiffness and Young's modulus (i.e. +20-51%) than observed in our study and were probably explained by the utilisation of higher exercise intensities and/or a different mode of exercise (i.e. traditional and flywheel resistance training) (2, 4, 35). It has been hypothesised that *high-intensity* exercises would submit the tendon to greater mechanical stress and strain, resulting in greater ultrastructural damage at the tendon-bone interface, which may be the main stimulus for tendon hypertrophy, and the reason why the patellar tendon tends to hypertrophy at the proximal and distal ends only (27, 44). In the present study, we reported an increase in stiffness and Young's modulus *in vivo* at four weeks but with no tendon hypertrophy, suggesting that the exercise intensity was not sufficient to induce microstructural changes necessary to promote tendon hypertrophy and/or training characteristics (e.g., training duration) were not optimal for this particular adaptation (47). Thus, the increase in stiffness appears to be attributed to changes in the material properties of the tendon (e.g. an increase in collagen content and/or greater cross-linking of collagen fibrils). Accordingly, Kaux et al. (24) reported a trend toward an increase (p=0.051) in patellar tendon Young's modulus, but not CSA, after five weeks' DR training in rats. Changes in extracellular

matrix protein synthesis, collagen fibril density, collagen crimping angle, proteoglycan synthesis, as well as water content might be responsible for changes in the tendon's mechanical properties following mechanical overloading (34, 49). Taken together, these results support the hypothesis that tendon mechanical adaptations *in vivo* may be influenced by exercise intensity (2, 4, 35) but, crucially, strain frequency, strain rate and strain duration may activate mechanotransduction pathways within the extracellular matrix, leading to tendon adaptations (5, 32).

Since patellar tendon loading during DR has never been measured directly *in situ* and may depend on several factors (e.g., running speed, slope, foot strike pattern, ground stiffness, body mass and ground reaction force), its exact magnitude remains unknown. Thus, it is unclear whether the changes we observed in tendon properties occurred in response to load magnitude and/or to repeated exposure to mechanical stress/strain in DR. The postulated link between adaptations in muscle and tendon is that the actual work performed by the former results in the stress experienced by the latter. Thus, it is commonly accepted that tendon adaptations to overloading are partly driven by the stress/strain resulting from muscular work (26). In the present study, strength gains did not appear to contribute to changes in patellar tendon stiffness and Young's modulus throughout the four-week DR training period. This suggests that patellar tendon properties did not change as a function of the increase in maximal tensile stress in our specific training programme. In line with this, Seynnes et al. (44) did not report a relationship between strength gains and changes in patellar tendon mechanical properties after nine weeks' resistance training in healthy, young, previously untrained adults. Thus, exercise-specific tendon mechanical stress seems to have no to minimal effect on tendon adaptations following short-term DR training.

Interestingly, acute *moderate-intensity*, *high-volume* exercises have been reported to promote collagen protein turnover (11, 31, 40), suggesting that this specific exercise modality

is a potent stimulator of tendon adaptations. Furthermore, patellar tendon collagen fractional synthetic rate (FSR) and *vastus lateralis* myofibrillar FSR follow a similar pattern regarding an increase in response to 1 h one-legged loaded kicking (38), demonstrating the high level of plasticity of both tissues to external loading. Accordingly, loading history could represent a major stimulus for chronic tendon adaptations. This hypothesis was supported by Seynnes et al. (44) who reported a strong correlation between the quadriceps femoris hypertrophy and patellar tendon stiffness after nine weeks' conventional resistance training in young men. In the present study, change in VL PCSA was correlated with changes in tendon stiffness and Young's modulus from 0 to 4 weeks. One could argue that the high volume of moderate-intensity eccentric contractions during DR (~160-170 strides per minute at DR_{15}, at a speed corresponding to 60-65% $\dot{V}O_{2max}$, according to our pilot data) may have contributed to the rapid changes in patellar tendon mechanical and material properties following the four-weeks' training. In line with this, Simonsen et al. (45) reported that swimming training (i.e. moderate-intensity, high-volume) increased the tensile strength of the rat Achilles tendon. In addition, some studies reported that endurance-trained male athletes have greater muscle-tendon unit stiffness (30) or tendon stiffness (48) compared to untrained individuals. It should be emphasized, however, that loading history is a complex mechanism underlying muscle-tendon adaptations to chronic exercise, and should not simply be interpreted as training volume. While the latter may play a major role in muscle-tendon adaptation (49), loading history could be influenced by several co-variables inherent to the activity. Although the loading history in DR likely differs from that derived from resistance training, it appears that tendon stiffness/modulus adaptions occur after both types of loading intervention and that the mechanisms underpinning these changes are not influenced by the exercise-specific loading history.

We do acknowledge some limitations with our study that could inform future research. For example, this study did not include a control group. However, participants were requested

not to change their weekly routine of physical activity and nutrition for the duration of this study. Moreover, although it was not possible to blind the lead researcher during data collection or analysis, the semi-automated tracking software used to measure the tendon force-elongation curves, which were subsequently used to measure tendon stiffness and Young's modulus, removed any subjectivity from the data analysis and risk of bias from the lead researcher. The lack of bias is also reflected in the lack of training effect regarding RMVC tendon force, tendon loading rate and tendon CSA. It should also be noted that tendon stiffness and modulus (the only tendon properties to demonstrate a significant training effect) were calculated using the last 20% of each participant's *lowest absolute* RMVC force. Therefore, the stiffness and modulus changes reported in this study were not affected by changes in MVC force. Furthermore, the test-retest reproducibility of our measurements was very good (with low CVs and high ICCs, Table 2). Related to this, Ekizos et al. (13) questioned the reliability of using ultrasound to measure tendon CSA but the test-retest reproducibility of our patellar tendon CSA measurements was excellent. Similarly, other studies have reported high patellar tendon CSA reliability using ultrasonography (29, 50), demonstrating that ultrasound can be used as an alternative to magnetic resonance imaging to assess tendon CSA. In addition, the patellar tendon moment arm was not measured in the present study and previously published data from a comparable population were used instead (14), thus potentially influencing the calculated tendon forces in this study. However, the sex difference in moment arm is minimal at 90° knee flexion (28), and baseline patellar tendon stiffness measured in the present study was comparable to previous reports in similar populations (35, 44). Furthermore, the patellar tendon moment arm is anatomically determined and does not change after chronic training (36), limiting potential errors regarding our within-subject comparisons. Furthermore, while we acknowledge that the relatively small sample size in our study may be perceived as a limitation, it was greater than the sample size estimated from our *a priori* power calculation, and it allowed

us to observe significant changes in patellar tendon properties, even after just four weeks' DR training. Finally, it should be emphasized that our results were obtained in a laboratory-designed training setting (i.e. recruiting a recreationally active population to perform specific, supervised DR training on a treadmill that enabled decline running). Therefore, it is uncertain whether comparable adaptations would be observed with a different population, terrain (e.g. road/off-road), and/or DR training characteristics (e.g. longer duration/more intense sessions, etc.). Further research is needed to describe the time course and amplitude of change regarding these influencing factors.

In conclusion, four weeks' *moderate-intensity, high-volume* DR training promoted patellar tendon adaptations in previously untrained, healthy adults. This translates into increased patellar tendon stiffness and Young's modulus at week four, but no tendon hypertrophy. Loading history (represented by muscle hypertrophy) appeared to contribute to changes in patellar tendon stiffness from 0 to 4 weeks, while increased mechanical stress seemed to have no significant effect on tendon adaptations. These novel data demonstrate how just four weeks' chronic *moderate-intensity, high-volume* eccentric exercise can elicit rapid tendon adaptations, which may have implications for both athletic and clinical populations.

PRACTICAL APPLICATIONS

It is well-known that tendon displays remarkable adaptability to increased loading, and that performing high-intensity resistance training is an effective strategy to promote tendon stiffening. However, high-intensity resistance exercise is not always feasible/desirable and other training modalities may be preferable, depending on the athlete/patient population. This study shows for the first time that *moderate-intensity, high-volume* eccentric training (in the form of downhill running, DR) is an effective stimulus to induce considerable patellar tendon adaptations (typically seen after high-intensity resistance training). Incorporating regular DR

exercise into their training programme could represent a novel and effective training modality for endurance runners seeking to stimulate their musculotendinous system, while maintaining their aerobic training volume. Indeed, using a more ecologically valid eccentric-based exercise (rather than high-intensity resistance training) to optimize patellar tendon stiffness should lead to a more effective transfer of force from the knee extensor muscles during each stride, thus improving running economy (1). In addition, the chronic use of DR may also lead to lower limb strengthening and muscle adaptations, as suggested by previous studies (6, 46). These adaptations could potentially have positive implications for athletic performance and injury risk reduction, as muscle weakness is known to be an injury risk factor (16, 52). It should be emphasized, however, that this implies the use of an adapted motorized treadmill and/or an established management of negative elevation in the training periodization of athletes (e.g., running on hilly terrains). Although the latter should elicit comparable patellar tendon adaptations to the results of our study, this still needs to be verified.

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Table 1. Downhill running (DR) training programme.

Table 2. Test-retest reproducibility data for assessments of patellar tendon (PT) morphological, mechanical, and material properties.

Table 3. Downhill running training-induced changes in patellar tendon properties. Data are mean ± SD.

Figure 1. Representative images from one participant, illustrating the patellar tendon (PT) force-elongation curve (A), the *vastus lateralis* muscle cross-sectional area (CSA) at 50% of the muscle length (B) and patellar tendon (CSA) at 25%, 50% and 75% tendon length (C).

Figure 2. Correlations between changes in patellar tendon (PT) mechanical and material properties, and changes in knee extensor isometric maximum voluntary torque (MVT) (A, C) and *vastus lateralis* physiological cross-sectional area (PCSA) (B, D). Pearson's correlation coefficient (r), the corresponding *p*-value and the 95% confidence bands were reported for each analysis.

Table 1. Downhill running (DR) training programme.

Training session	Grade-specific running duration (min)			Total running duration (min)	Negative elevations (m)
	DR_5	DR_{10}	DR_{15}		
n°1	5	5	5	15	304 ± 44
n°2	10	5	5	20	347 ± 50
n°3	5	10	5	20	402 ± 59
n°4	5	8	7	20	441 ± 64
n°5	10	10	5	25	446 ± 65
n°6	5	5	5	15	304 ± 44
n°7	5	7	13	25	604 ± 87
n°8	7	7	13	27	621 ± 89
n°9	7	8	15	30	706 ± 101
n°10	5	10	15	30	729 ± 105

DR_5: downhill running slope = -5°; DR_{10}: downhill running slope = -10°; DR_{15}: downhill running slope = -15°

Table 2. Test-retest reproducibility data for assessments of patellar tendon (PT) morphological, mechanical and material properties.

	Typical error (95% CI)	CV (%)	ICC (95% CI)
Morphological PT properties			
Mean CSA (mm^2)	1.23 (0.79-2.70) mm^2	1.2	0.988 (0.915 -0.998)
CSA 25% tendon length	1.26 (0.81-2.78) mm^2	1.7	0.989 (0.926-0.998)
CSA 50% tendon length	1.30 (0.84-2.87) mm^2	1.3	0.982 (0.882-0.998)
CSA 75% tendon length	1.94 (1.25-4.27) mm^2	1.7	0.975 (0.834-0.996)
Resting tendon length (mm)	1.03 (0.66-2.26) mm	2.3	0.979 (0.858-0.997)
Mechanical PT properties			
MVT (N·m)	4.81 (3.10-10.60) N·m	1.84	0.998 (0.984-1.000)
RMVC tendon force (N)	240 (155-529) N	4.4	0.983 (0.888-0.998)
Elongation (mm)	0.26 (0.17-0.57) mm	6.6	0.943 (0.653-0.992)
Stiffness ($N \cdot mm^{-1}$)	98 (63-216) $N \cdot mm^{-1}$	4.1	0.995 (0.964-0.999)
Strain (%)	0.39 (0.25-0.87) %	4.8	0.969 (0.802-0.996)
Stress (MPa)	0.80 (0.45-2.99) MPa	1.3	0.983 (0.397-1.000)

Material PT properties			
Young's modulus (GPa)	0.06 (0.03-0.22) GPa	4.96	0.992 (0.670-1.000)

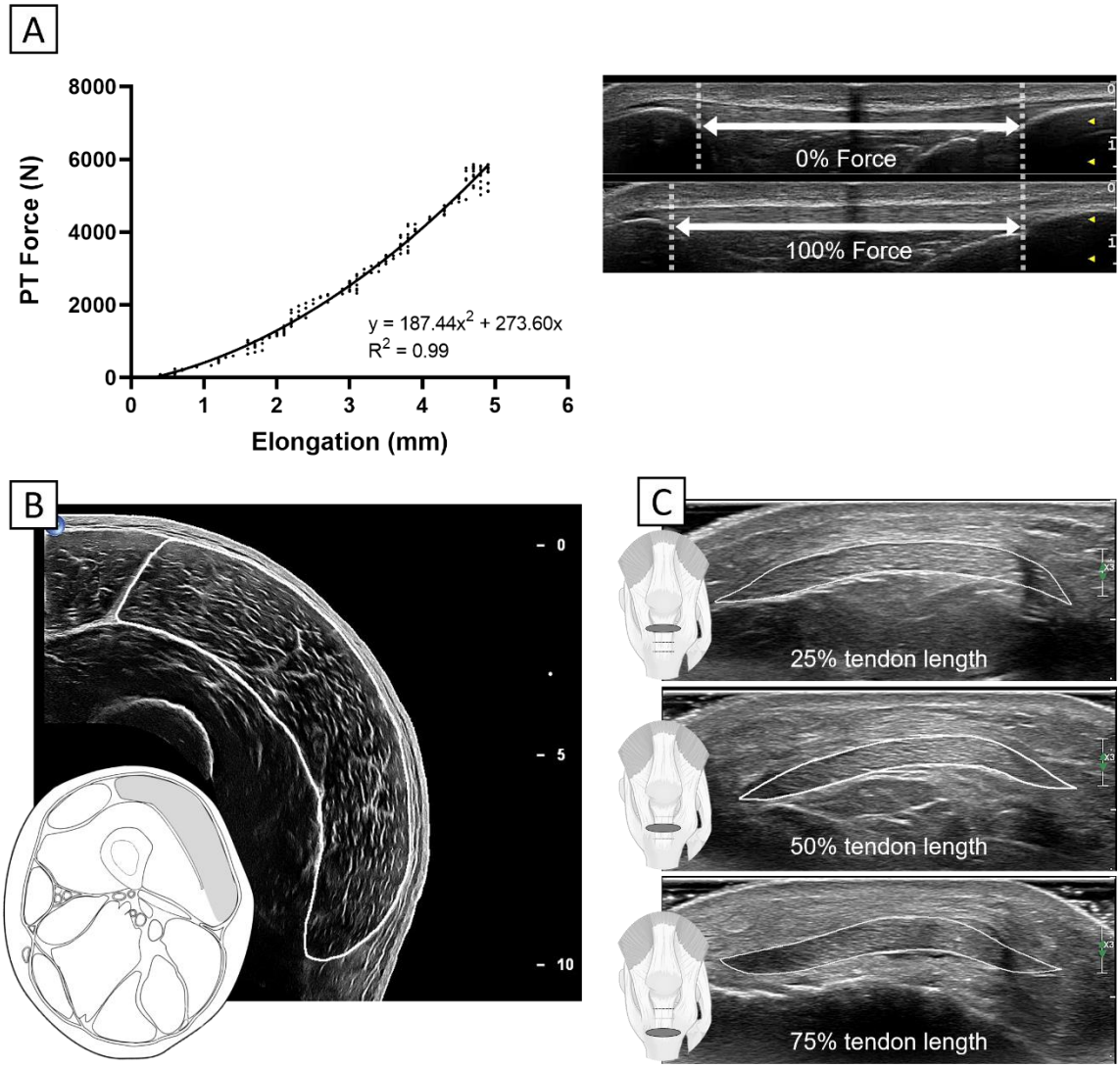
CSA: cross-sectional area; MVT: maximal voluntary isometric torque; RMVC: ramped maximal voluntary isometric contraction.

Table 3. Downhill running training-induced changes in patellar tendon properties. Data are mean ± SD.

	0-weeks	2-weeks	4-weeks	ANOVA
Morphological properties				
Mean CSA (mm^2)	98.42 ± 11.75	98.64 ± 12.50	98.56 ± 11.95	F (2, 21) = 2.102; χ^2 = 163; p = 0.147
CSA 25% tendon length (mm^2)	92.06 ± 11.41	93.27 ± 11.50	92.24 ± 11.45	F (2, 21) = 1.728; χ^2 = 135; p = 0.202
CSA 50% tendon length (mm^2)	96.08 ± 11.18	96.59 ± 11.88	96.10 ± 11.50	F (2, 21) = 0.282; χ^2 = 138; p = 0.757
CSA 75% tendon length (mm^2)	107.12 ± 14.65	106.01 ± 15.07	107.34 ± 14.89	F (2, 21) = 1.030; χ^2 = 141; p = 0.374
Resting tendon length (mm)	48.39 ± 6.70	48.55 ± 6.95	48.64 ± 7.07	F (2, 22) = 393.8; p = 0.593
Mechanical properties				
MVT (N·m)	241 ± 71	254 ± 92	**262 ± 89***	F (2, 22) = 4.044; ***p* = 0.032**
RMVC tendon force (N)	4310 ± 1429	4456 ± 1782	4817 ± 1712	F (2, 20) = 2.608; p = 0.099
RMVC tendon force / MVT (%)	89.58 ± 7.64	88.15 ± 23.29	92.47 ± 22.19	F (2, 20) = 0.298; p = 0.746
Loading rates ($N \cdot s^{-1}$)	618 ± 233	640 ± 304	674 ± 272	F (2, 20) = 0.414; p = 0.667
Elongation (mm)	4.52 ± 1.07	4.18 ± 0.60	4.45 ± 0.66	F (2, 20) = 1.362; p = 0.279
Stiffness ($N \cdot mm^{-1}$)	1704 ± 491	1699 ± 525	**1815 ± 524***	F (2, 20) = 4.247; ***p* = 0.029**
Strain (%)	9.47 ± 2.21	8.74 ± 1.04	9.34 ± 1.63	F (2, 20) = 1.367; p = 0.277

Stress (MPa)	40.02 ± 11.27	40.35 ± 11.73	39.96 ± 11.26	F (2, 19) = 2.398; χ^2 = 177; p = 0.118
Material properties				
Young's modulus (GPa)	0.83 ± 0.23	0.84 ± 0.27	**0.89 ± 0.26***	F (2, 19) = 3.886; χ^2 = 46.43, ***p* = 0.039**

CSA: cross-sectional area; MVT: maximal voluntary isometric torque; RMVC: ramped maximal voluntary isometric contraction. * Different to 0-weeks ($p < 0.05$).



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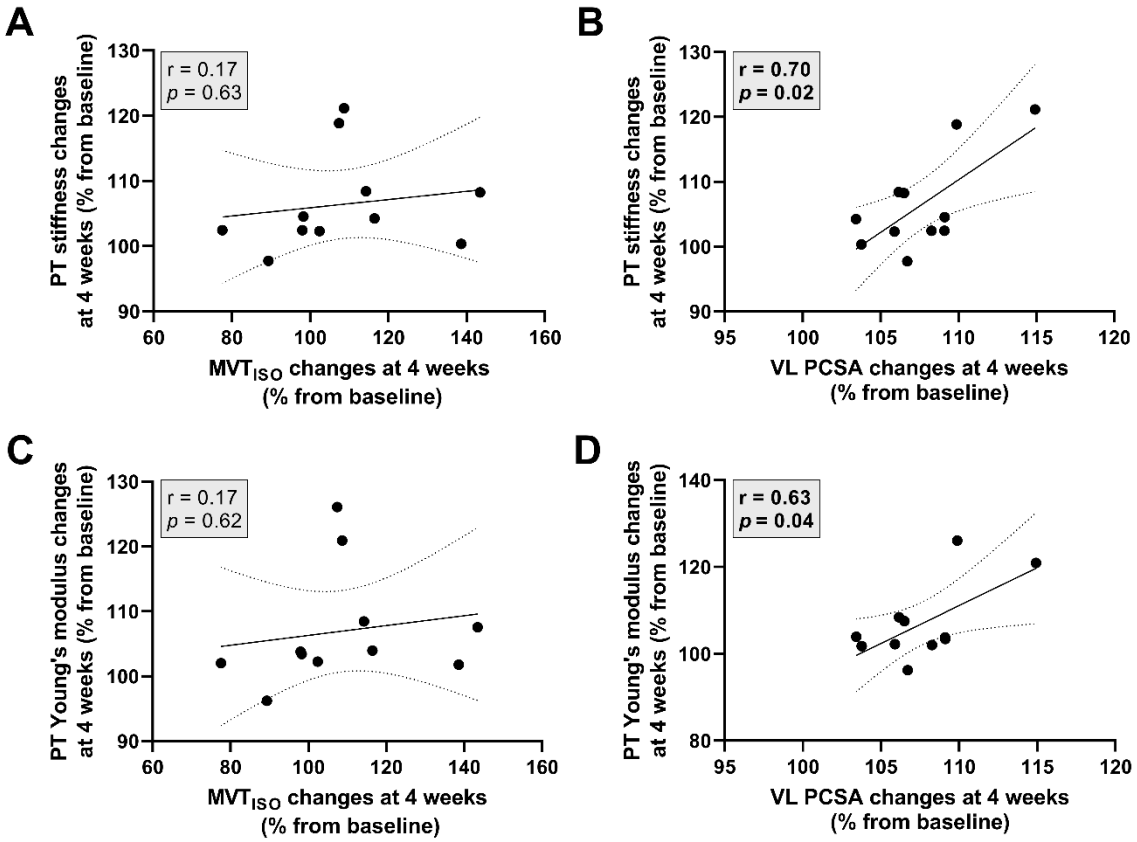

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