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Evidence of different sensitivity of muscle and tendon to mechano-metabolic stimuli

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Abstract

This study aimed to examine the temporal dynamics of muscle–tendon adaptation and whether differences between their sensitivity to mechano-metabolic stimuli would lead to non-uniform changes within the triceps surae (TS) muscle–tendon unit (MTU). Twelve young adults completed a 12-week training intervention of unilateral isometric cyclic plantarflexion contractions at 80% of maximal voluntary contraction until failure to induce a high TS activity and hence metabolic stress. Each participant trained one limb at a short (plantarflexed position, 115°: PF) and the other at a long (dorsiflexed position, 85°: DF) MTU length to vary the mechanical load. MTU mechanical, morphological, and material properties were assessed biweekly via simultaneous ultrasonography–dynamometry and magnetic resonance imaging. Our hypothesis that tendon would be more sensitive to the operating magnitude of tendon strain but less to metabolic stress exercise was confirmed as tendon stiffness, Young's modulus, and tendon size were only increased in the DF condition following the intervention. The PF leg demonstrated a continuous increment in maximal AT strain (i.e., higher mechanical demand) over time along with lack of adaptation in its biomechanical properties. The premise that skeletal muscle adapts at a higher rate than tendon and does not require high mechanical load to hypertrophy or increase its force potential during exercise was verified as the adaptive changes in morphological and mechanical properties of the muscle did not differ between DF and PF. Such differences in muscle–tendon sensitivity to mechano-metabolic stimuli may temporarily increase MTU imbalances that could have implications for the risk of tendon overuse injury.

KEYWORDS
mechano-metabolic stimuli, muscle–tendon adaptation, tendon strain, training to failure

1 | INTRODUCTION

Tendons are an integral part of muscle–tendon units (MTUs) and their primary function is to transmit muscle forces to the skeletal system to produce motion. Due to their viscoelastic nature, tendons respond to mechanical loading with deformation and can thereby store and release strain energy during muscular contractions.[1] Taking into account that the ultimate tendon strain has been reported to be rather constant,[2] an increase in the operated tendon strain during muscular contractions indicates to a higher mechanical demand for the tendon.[3] This has been proposed as an important mechanical risk factor for the development of tendon overuse injuries and failure.[4,5] Hence, an imbalance between muscle strength gains and changes in tendon stiffness (i.e., resistance to deformation when subjected to an applied force) may disrupt the integrity of the MTU to meet the functional demand. Identifying potential risk factors which compromise the homeostasis of human tendon may therefore be of great importance to prevent predisposition to overuse injuries within the MTU.

Muscle is known to be highly sensitive to mechanical loading and can alter its morphological and neuromechanical properties due to disuses, immobilization or exercise-related interventions.[6] Strength gains induced by exercise are often results of combined central or neural factors as well as peripheral morphological adaptive changes within the muscle.[6] Morphological adaptations include predominantly increased muscle thickness and pennation angle, both yielding changes in physiological cross-sectional area.[7] It is well established that muscle responds well to a variety of mechanical loading modalities when a specific overall training volume is exceeded without the requirement to incorporate high training loads.[8] For example, it has been shown that muscle protein synthesis rate, muscle volume, and strength increase in a similar manner with exercise that reaches volitional (muscular) failure under both low and heavy loading (i.e., 30% vs. 80% of maximal strength).[9] The effect of such muscular contractions to failure on muscle strength gains is supported by evidence from studies which observed the accumulation of by-products such as lactate, hydrogen ions,[10] inorganic phosphate, and mitogen-activated protein kinase, all of which may induce an anabolic signal and hence muscle hypertrophy.[11]

In contrast to muscle, tendon is reported to be sensitive to the magnitude of mechanical demand and hence to the operating tendon strain during muscular contractions.[12,13] Tendon has been consistently shown to adapt effectively to a strain of 4.5% to 6.5%,[13] a level which can only be achieved under a relatively high magnitude of mechanical loading (i.e., muscular contraction). The experienced tendon strain is transferred to its cellular cytoskeleton via the extracellular matrix causing intracellular signaling of gene and growth factor expression which in turn increase collagen and matrix protein synthesis.[12] In addition, previous animal studies have shown a loading-induced higher production of enzymes that mediate collagen cross-linking,[14] which has also been considered to modulate the adaptations in human tendons following resistance exercise using high-loading profiles.[15] Furthermore, tendon's adaptive responsiveness to increased mechanical loading interventions seems to be at a much slower rate compared with muscle,[16] potentially due to their lower cell to overall dry mass ratio, vascularization, metabolism and lower tissue turnover.[17,18] Thus, we may suggest that the adaptation within the MTU could potentially be imbalanced during exercise-related interventions due to differences in sensitivity to mechano-metabolic stimuli between muscle and tendon. However, based on our knowledge, the effects of high metabolic stress or physiological demand caused by exercising to muscular failure with low magnitudes of mechanical loading on the temporal dynamics of muscle and tendon adaptation is currently unknown.

The purpose of this study was to test the hypothesis that human tendon and muscle have a different mechano-metabolic sensitivity with respect to the rate and magnitude of adaptive changes (due to exercise) leading to non-uniform adaptation within the MTU. Specifically, based on earlier reports[9,19] we expected that both low- and high-load muscular contractions until failure would result in similar alterations in muscle thickness, pennation angle, and muscle strength while tendon adaptation would be more sensitive to the magnitude (i.e., higher) of mechanical load. Hence, in the current study we aimed to induce high metabolic stress and physiological demand within the triceps surae (TS) MTU by implementing cyclic contractions until failure over 12 weeks with different levels of mechanical loading by modifying the operating length and consequently force potential of the contractile element (plantarflexed vs. dorsiflexed position). To our knowledge, this is the first in vivo study addressing human muscle and tendon responsiveness to mechano-metabolic stimuli over time and the findings may provide relevant information for the (un)balanced adaptation of human muscle and tendon to exercise.

2 | METHODS

2.1 | Ethical approval

The study was approved by the responsible ethics committee (London South Bank University Applied Sciences Ethics Panel, Application no. ETH2021-0179) and all procedures were performed in accordance with standards set by the Declaration of Helsinki, except for registration in a database. Written informed consent was obtained from all participants prior any measurements.

2.2 | Participants and experimental design

Twelve healthy young adults (males $n=9$; females $n=3$) voluntarily participated in this study (age 27 ± 5 years; body mass 75.6 ± 12.0 kg; body height 1.75 ± 0.08 m; mean and standard deviation). All participants were physically active (about 3 times of 1 h per week in non-competitive sports) with no previous musculoskeletal injuries at the lower extremity over the past 6 months or any neurological disorder or undergoing any drug medication during the experiments. Further exclusion criteria included any previous Achilles tendon (AT) rupture or tendinopathy; and any specific targeted calf training exercise such as resistance training in the last 6 months to decrease any potential bias effects caused by additional mechanical loading exercise.

In order to examine the sensitivity of muscle and tendon as well as the temporal dynamics of their adaptations to mechano-metabolic stimuli, a 12-week training intervention of 3 weekly sessions of cyclic fixed-end plantarflexion (three sets at 80% of maximal voluntary contraction [MVC] of 3 s loading and 3 s unloading contractions until failure) was implemented. Baseline (PRE), during (W01–W11), and post the 12 weeks (W12) of strength training intervention, TS MTU mechanical (muscle strength, tendon stiffness, maximal tendon strain), morphological (muscle thickness and pennation angle of all three TS compartments, length, CSA, and micromorphology of the free AT) and tendon's material properties (Young's modulus) were assessed for each individual via dynamometry, magnetic resonance imaging (MRI), and ultrasonography (Figure 1). Baseline testing (PRE) for the mechanical properties of the MTU and muscle architecture (via ultrasonography) was conducted twice, approximately 10 (PRE1) and 5 (PRE2) days before the beginning of the intervention to determine the reliability of the analyzed parameters and consider the biological variation of muscle and tendon properties. The means of all MTU variables assessed in the two baseline testing sessions (PRE1 and PRE2) were considered for further analysis (PRE baseline values).

2.3 | Analysis of TS MTU mechanical and material properties

Maximal ankle plantarflexion moment, maximal tendon strain, and stiffness were assessed for each leg every 2 weeks (Figure 1) using synchronized ultrasonography and dynamometry on a custom-made device. The participants were seated with their knee joint set at 90° and ankle joint at 85°, and their foot placed on a strain gauge-type dynamometer (1000 Hz; TEMULAB®, Protendon GmbH & Co. KG, Aachen, Germany). The foot was positioned on the dynamometer with the midpoint of its lateral

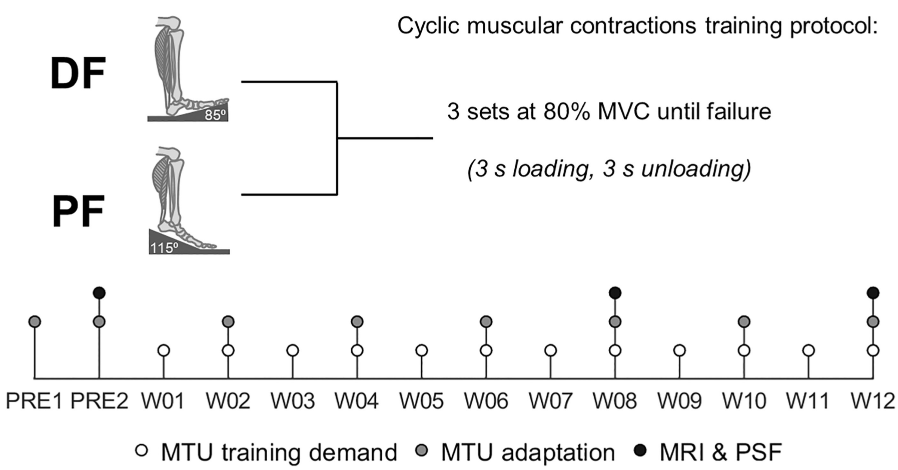

FIGURE 1 Experimental design and protocol. Muscle–tendon unit (MTU) training demand: Three sets at 80% MVC of cyclic 3 s loading and 3 s unloading contractions until failure while ankle joint moment and maximum tendon strain in each leg were assessed weekly during training. MTU adaptation: biweekly assessment of MTU mechanical and morphological (via ultrasonography) properties. Additional magnetic resonance scans (MRI) and peak spatial frequency (PSF) analysis using ultrasonography was conducted at PRE2, W08 (Week 8 post-start of the intervention) and at W12 (Week 12 post-start of the intervention) to assess potential adaptive changes in Achilles tendon morphological and material properties. DF, dorsiflexed joint angle configuration; MVC, maximum voluntary isometric contraction; PF, plantarflexed joint angle configuration.

malleolus in line with the dynamometer's axis of rotation. This standardized positioning for each participant at each measurement time point was achieved using a laser-guided electrical potentiometer system.[20] In order to "precondition" the tendon, participants performed an individual warm-up before the measurement followed by a standardized warm-up routine for 2–3 min of submaximal and three maximal isometric contractions visually guided by the TEMULAB® software on a computer screen. Then, participants were asked to perform several voluntary isometric ankle plantarflexion contractions at various joint moment levels to assess the maximal ankle plantarflexion moment as well as the force–elongation relationship of the tendon during the loading phase. First, the participants performed two isometric ankle plantarflexion MVCs while being verbally encouraged by the investigators. The maximal joint moment was used to perform further three visually guided sustained contractions at 30%, 50%, and 80% of the MVC (each of the three target joint moment levels was reached with a ramp contraction within 2–3 s followed by steady state) for the analysis of the AT force–elongation relationship. The resultant ankle joint moment was calculated via inverse dynamics and taking into consideration the gravitational moments.[20] Due to the relatively low ankle joint angular rotation (on average less than three degrees) and hence anterior translation of the malleoli in relation to the axis of rotation of the force plate during MVCs, the obtained moments at the force plate can be considered equal to the ankle joint moments. Subsequently, the AT force was calculated by dividing the resultant joint moment by the individual tendon moment arm (see "Analysis of AT morphological properties").

In order to assess tendon elongation during contractions an ultrasound (US) probe (27 Hz; MyLab™Five, Esaote, Genoa, Italy) was placed on the myotendinous junction (MTJ) of the gastrocnemius medialis (GM) muscle using a rigid custom-made casing and fixed to the shank with adjustable straps. A laser-guided electrical potentiometer embedded in the dynamometer system (TEMULAB®) registered the exact location of US probe fixation on the shank and supplementarily allowed a constant probe positioning for each participant and leg over the course of all measurement time points. An adhesive tape placed on the skin close to the GM MTJ was used to register any motion of the probe during the contractions.[21] The GM MTJ displacement was digitized at rest (0% MVC) and in the mentioned three sustained contractions (steadily held for 3 s) at the target ankle joint moments (30%, 50%, 80% MVC) using TEMULAB® software (Protendon GmbH & Co. KG, Aachen, Germany). Failure to hold a steady state for 3 s within range of ±5% of the target moment prompted a repetition of the specific trial. Due to the inability of most subjects to generate maximal forces over several seconds tendon elongation at maximal force (100%) was assessed using a linear extrapolation of the elongation between 50% and 80% target joint moments. As the linearity of tendon force and elongation between 50% and 100% MVC has been demonstrated to be reasonably high (coefficient of determination $R^2 = 0.98–0.99$),[22] any potential errors would perhaps marginally affect the estimated maximal tendon elongation and strain, but not in relative terms as the same approach was used between conditions and all measurement time points. The effect of potential ankle joint angular rotation on the measured elongation of the tendon during contraction was taken into account using the product of the ankle joint angle changes and the individual AT moment arm taken from the MRI scans (see "Analysis of AT morphological properties"). The changes in ankle joint angle were calculated as the inverse tangent of the ratio of the heel lift (measured with a potentiometer) to the distance between the ankle joint axis and the head of the fifth metatarsal bone.[20] AT stiffness was calculated as the ratio of the respective changes in calculated tendon force to the resultant tendon elongation between 30% and 80% of maximum tendon force. In order to assess maximal tendon strain during MVCs, the resultant elongation at maximal force (100%) was normalized to the tendon length, which was determined before muscular contractions as the distance between the GM MTJ and the most proximal point of the tuber calcanei (both detected in advance using ultrasonography). The above MTU assessment was conducted 10 and 5 days before the start of the intervention (PRE1 and PRE2), every 2 weeks during (W02, W04, W06, W08, W10) and post-training phase (W12; see Figure 1). Finally, AT elastic modulus (Young's modulus) was calculated as the ratio of changes in tendon stress and AT strain from 30% to 80% of the maximal AT stress at baseline (PRE2), at W08 and at the end of the intervention (W12; see Figure 1). The AT stress was determined as the ratio between the calculated AT force and the average CSA along the free AT determined using MRI (see "Analysis of AT morphological properties"). The MRI measurement at W08 was chosen as the second timepoint as we expected greater relative muscle hypertrophy and strength gains in comparison with tendon material and morphological alterations[16] potentially leading to increased tendon strain values during MVCs.

2.4 | Analysis of TS muscle morphological properties

The thickness and fascicle pennation angle of the three compartments of TS muscle (m. gastrocnemius medialis and lateralis, GM and GL; m. soleus, SO) were investigated via ultrasonography at baseline (PRE1 and PRE2) and every 2 weeks (Figure 1). Firstly, the participant laid prone on a massage table with hips and knees fully extended and the ankle joint fixed at 90° in a resting state (no active contraction). The shank length was measured as the distance between the tuberositas calcanei and the tibial plateau, both determined and marked using a 50 mm linear array ultrasound probe (MyLab™Five, Esaote; 29 Hz). Based on the measured shank length the probe was then positioned at the height where previously the maximal CSA for all three compartments of the TS has been reported for young individuals (GM≈80%, GL≈84% and SO≈67%).[23] A potentiometer fitted in a rigid custom-made plastic case was attached to the probe to record the pressure applied during each scan. The probe positioning heights and applied pressure were kept constant between all the measurement time points. Three US images were recorded for each muscle and further digitized by a single investigator using custom-made scripts in MATLAB 2022a (MathWorks, Inc., Natick, MA, USA). From the US images pennation angle was determined as the angle between a muscle fascicle and the lower aponeurosis, whereas the muscle thickness was assessed via the perpendicular distance between the upper and lower aponeuroses.[22] For each muscle and testing session, the mean values of the three sequences were considered for further analysis.

2.5 | Analysis of AT morphological properties

The morphological properties of the free AT were examined using MRI at baseline (PRE2), at W08 and post-intervention (W12; see Figure 1). The participants laid supine in a 3 Tesla MRI scanner (MAGNETOM Skyra; SIEMENS, Munich, Germany) with the hip and knee fully extended and the ankle joint fixed in an ankle coil set at neutral 90°. T1-weighted MRI Turbo Spin Echo (TSE) sequences were acquired in the transverse and sagittal planes using the following settings: acquisition matrix 320 × 224, time of echo (TE) 11 ms, time of repetition (TR) 1190 ms and slice thickness 3 mm. An image segmentation software ITK-SNAP was used to manually outline the boundaries of the AT in every transversal image along the length of the free tendon (from most proximal attachment on the tuber calcanei to most distal aspect of the soleus muscle)[24] by a single investigator blind to the leg and recording time point. The acquired segmentations of the whole AT were exported as a surface mesh and further processed using custom scripts in MATLAB. The curved path through the centroids (calculated using Delaunay triangulation) of every segmented CSA between the two landmarks stated earlier was used to determine the length of the free AT. Region-specific changes in AT were examined by calculating the CSA in every 10% intervals along the free AT. In addition, individual AT moment arm was calculated as the perpendicular distance from the estimated ankle joint's center of rotation (average position between the most prominent apex of medial and lateral malleolus) to the line of force at the corresponding centroid of the AT.[25]

In addition to the above MRI measurements, tendinous tissue micromorphology was assessed based on a spatial frequency analysis of ultrasound images of the distal, mid, and proximal part of the free AT at PRE, W08, and W12. Different regions of the free AT were assessed as exercise or pathology can potentially cause a region-specific adaptation in tendon's morphology, for example, Kulig et al.[26] found pathology-related effects only at the proximal part of the free patellar tendon. The participants were positioned prone with their knee and hip joints fully extended and ankle joint set at 90°. The linear array ultrasound probe (SL1543; MyLab™Five, Esaote, Genoa, Italy; 13 MHz; depth: 22 mm) was placed over the AT along its longitudinal axis at 25% (distal), 50% (mid), and 75% (proximal) part of the free tendon length (from most proximal attachment on the tuber calcanei to most distal aspect of the soleus muscle) of each participant and leg.

For the analysis of three recorded images at each height, a polygonal region of interest (ROI) was defined in a custom-written MATLAB interface (version R2021b; MathWorks) with its length corresponding to 25% of the free tendon length of each participant and its height covering the full thickness of the tendon. Within this ROI, all kernels of 32 × 32 pixels were analyzed as suggested by Bashford et al.[27] using a 2D Fast Fourier transform (FFT) and a high-pass filter with a radial frequency response and half-power cutoff frequency of 1.23 mm^{-1}. In order to increase the frequency resolution, the filtered kernels were zero-padded in both directions to a size of 128 × 128 pixels. The average distance of the peak spatial frequency (PSF) from the spectral origin in the frequency spectrum of all possible kernels was calculated and the mean PSF of all three trials at each height was used for further analysis as a measure of the packing density and alignment of the collagen bundles.[27]

2.6 | TS MTU cyclic fatiguing exercise intervention over 12 weeks

All participants performed the mechano-metabolic loading exercise intervention in a controlled laboratory setting three times a week, at the same time of day within a time window of 2.5 h, with successive sessions separated by at least 48 h. A custom-made training device equipped with two strain gauge-type force plates (1000 Hz) for both legs and a visual feedback system developed with LabVIEW 2013 SP1 (National Instruments, Austin, TX, USA) was used throughout the entire intervention by all participants (see also Epro et al.[28]). In each session, participants were seated on a rigid height-adjustable stool with their hip and knee fixed at 90° and feet placed on the dynamometers. As for the assessment of TS MTU mechanical properties, the malleolus lateralis defined as the ankle joint axis was positioned for each leg in line with the dynamometer's axis of rotation using a system of adjustable (in the sagittal plane) foot placement. Subsequently, a standardized warm-up of 2–3 min of submaximal isometric plantarflexion contractions was performed followed by two unilateral MVCs from which the participant's 80% training level of that session was calculated. By this means, every session's load was adjusted and progressively increased based on the individual's muscle strength for each leg throughout the intervention. A training session consisted of three sets of unilateral isometric plantarflexion contractions (3 s loading, 3 s unloading) at 80% MVC until failure to induce high metabolic stress.[29] Failure was defined as the time point when the participant could no longer reach higher than ~75% MVC on the live visual feedback or could not hold a contraction for all 3 s as required. The preceding valid repetition was regarded as the maximal number of repetitions for the respective set. In each participant, one leg was trained at a short (PF; 115° ankle joint angle; tibia perpendicular to the shank: 90°) and the other at a long (DF; 85° ankle joint angle) MTU length to achieve low and high levels of muscle mechanical load and tendon strain, respectively. Previous investigations[22] have confirmed that shortening the TS MTU by using a more plantarflexed joint angle configuration reduces the force potential of the TS due to the force–length relationship and will in turn reduce the experienced tendon strain magnitude during cyclic loading exercise. In order to evaluate this in the current study, we monitored the experienced tendon strain and generated joint moments in each training week across the 12-week exercise intervention (for more details please see next paragraph). Two minutes of rest were allowed between each set and another 15–20 min were given before the second leg was trained to prevent any potential detrimental effect of central nervous system fatigue on the performance. Furthermore, the leg to start with the training was changed in each session over the entire 12-week period of exercise. The target ankle joint angles for DF and PF positions were achieved using two wooden blocks (one of −5° and the other 25° of inclination) attached to the flat surface of the dynamometers foot plates (Figure 1). Training was performed barefoot to reduce potential effects of shoe geometry on TS MTU length during training. In order to avoid slipping of the feet on the wooden blocks, individual non-slippery socks were provided to the participants. In addition, since the PF-trained leg had a high inclination angle, a custom-made fixation, assembled from the material of a ski boot buckle-catch system, was applied around the foot and the force plate platform to further reduce any foot movement during contractions. At least one investigator was supervising each training session to provide verbal encouragement for maximal performance and ensure the highest attention to the task.

2.7 | Estimation of muscle activation volume, mechanical loading, and tendon strain during training

In order to assess whether the cyclic loading exercise until failure induced a similar physiological demand between short (PF) and long (DF) MTU lengths, we measured TS muscle electromyographic (EMG) activity 2 weeks prior to the start of the intervention. For this reason, in a randomized cross-over study design all participants underwent a single training session (three sets of 80% MVC until failure) in both DF and PF conditions while recording the EMG activity of all three TS compartments (GM, GL, and SO) using bipolar EMG leadoffs, with pre-amplification (bandwidth 10–500 Hz) and adhesive surface electrodes (Ag/AgCl; BlueSensor, Ambu, Copenhagen, Denmark). The EMG signal (Figure 2A) was recorded using the Qualisys system (QTM v2022; Qualisys, Gothenburg, Sweden) at 1000 Hz sampling frequency. In order to compare TS EMG recordings between the two conditions, training at DF and PF was performed unilaterally within one session and hence no electrode replacement was required during testing (left and right legs were randomized across subjects). Furthermore, to reduce any potential central fatiguing effects on EMG outcome variables and muscle failure resistance, a resting period of minimum 3 h was given between the two conditions. For each muscle and exercise set, the integral of the EMG activation (GM, GL, and SO) over time (total duration of the exercise until failure) was calculated from the full wave rectified and filtered data (using a Butterworth low pass filter). Finally, for each TS compartment (GM, GL, and SO) and condition (DF and PF) the total activation volume of the three analyzed training sets was considered for further analysis.

Furthermore, in the last training session of each week over the 12-week intervention, experienced AT strain magnitude during exercise was assessed in the first three cyclic contractions (80% of MVC) for each leg (DF and PF). For this reason, the displacement of the GM MTJ during the contractions was tracked using a firmly fitted 60 mm linear array ultrasound probe (7 MHz; ArtUs EXT-1H, TELEMED UAB, Vilnius, Lithuania). The inevitable ankle joint angle changes in the sagittal plane during

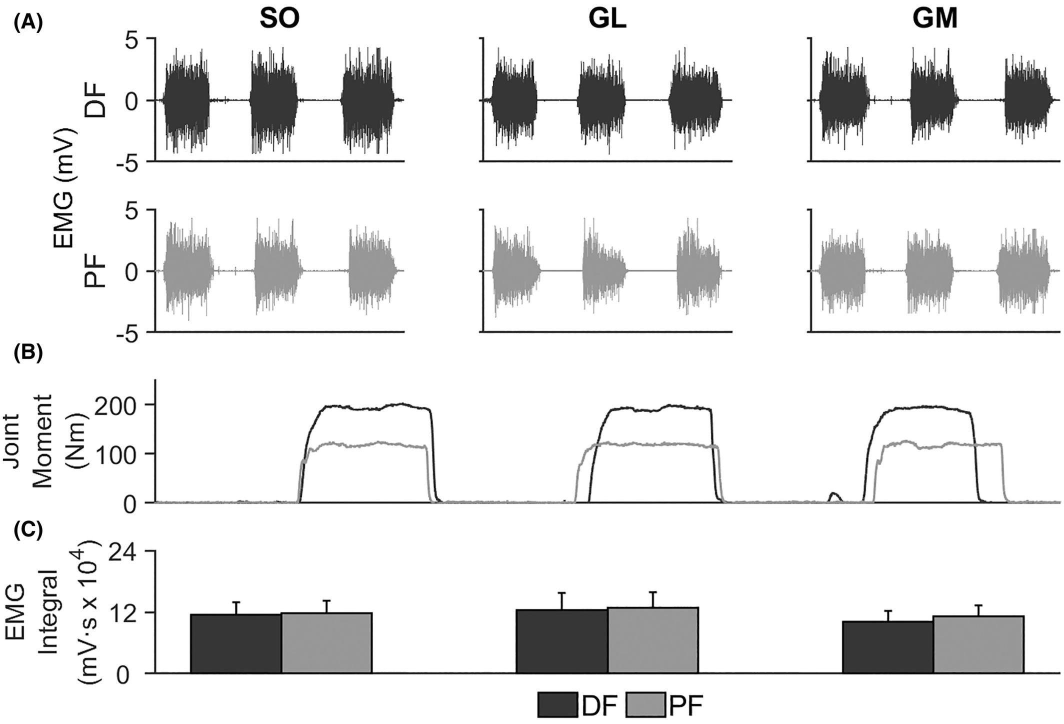

FIGURE 2 (A) Example of raw EMG recordings of each triceps surae compartment (GM: m. gastrocnemius medialis; GL: m. gastrocnemius lateralis: SO: m. soleus) and (B) corresponding joint moments during unilateral cyclic contractions until failure in dorsiflexed (DF) and plantarflexed (PF) joint angle configuration (three cyclic loading contractions are shown for each leg). (C) Using the data from (A) the integral of the EMG recordings over time was assessed to estimate TS muscle activity of all three compartments during cyclic loading exercise until failure. No significant differences between DF and PF were detected for GM, GL, and SO.

contractions were assessed by using a digital video camera (EOS 250D, Canon Inc., Tokyo, Japan) placed orthogonal to the foot dynamometer system. Both the measured tendon elongation from the ultrasound videos and the ankle joint rotation from the camera videos were manually digitized using the software Tracker (v6.0.9, Open Source Physics). The effect of potential ankle joint angular rotation on the measured elongation of the tendon during contraction was taken into account using the product of the ankle joint angle changes and the individual AT moment arm taken from the MRI scans. In order to assess the operating tendon strain during cyclic loading exercise at 80% of the MVC, the resultant tendon elongation was normalized to the corresponding tendon length for DF and PF legs before muscular contraction using flexible tape and ultrasonography in every testing session. The experienced magnitude of muscle mechanical loading during cyclic fatiguing loading exercise over the 12-week intervention was estimated by calculating the joint moments at 80% during the same contractions as for the tendon strain assessment using the two implemented force plates and the digital video camera. The average values in peak joint moment and experienced peak tendon strain of the first three cyclic contractions at 80% MVC were considered for further analysis. Finally, in order to estimate total mechanical loading volume of the MTU in DF and PF condition, the integral of the generated ankle joint moment over time during training was calculated including all sessions and repetitions across the entire 12-week intervention period.

2.8 | Statistics

The normality of distribution and homogeneity of variance of the data was confirmed using the Shapiro–Wilk and Levene test. Two-way repeated-measures ANOVAs with leg (DF vs. PF) as between-factor and corresponding measurement time points as within-factor were used for the following: AT morphology from MRI (five variables = mean CSA, free AT length, PSF at 25%, 50%, and 75% AT length), US muscle architecture (6 variables = 3 muscles [GM, GL, SO] × 2 properties [muscle thickness, pennation angle]), MTU mechanical properties (6 variables = maximal joint moment [(i) biweekly testing session, (ii) weekly training session], AT stiffness [testing], maximal AT strain [(i) testing, (ii) training], AT Young's modulus [testing]) and average number of repetitions in the three sets performed to failure. To identify potential region-specific effects on AT CSA, a three-way repeated-measures ANOVA (with leg as between-factor and both time and interval as within-factors) was used to statistically analyze every 10% interval along the entire length of the free AT. A Bonferroni post-hoc comparison was performed in the case of a detected significant main effect or interaction. Paired samples *t*-tests were used to examine differences in EMG integrals of the three TS muscle compartments during training between DF and PF conditions. Furthermore, paired samples *t*-tests were used to check for potential differences in total mechanical loading volume during training (integral of joint moment over time) between DF and PF. All statistical analyses and calculations were performed on MATLAB or SPSS statistics software (v27, IBM, Armonk, NY, USA) and the level of significance was set at $\alpha=0.05$. All results provided in the text, tables, and figures are presented as means and standard deviation (SD) and *n* is defined as the number of participants.

3 | RESULTS

3.1 | TS MTU mechano-metabolic demand during training

On average (average values over the 12 weeks of exercise intervention) the experienced magnitude of joint moment and operating AT strain during training were 204 ± 33 Nm and $5.7\pm0.2\%$ for the DF, and 126 ± 34 Nm and $2.0\pm0.1\%$ for the PF leg, respectively (Figure 3). When considering all analyzed weekly data points over the 12 weeks of exercise there was a significant time effect ($F_{3.983,43.810}=45.944$, $p<0.001$) and leg effect ($F_{1,11}=97.466$, $p<0.001$) on mechanical load (i.e., experienced magnitude of joint moment during training) and a time × leg interaction on the experienced magnitude of AT strain ($F_{1.833,40.333}=27.762$, $p<0.001$) during training. The post-hoc analyses revealed significantly higher max joint moment and tendon strain values for the DF in comparison with the PF leg independent of the 12 analyzed timepoints ($p<0.001$). The operating AT strain during contractions until failure of 80% of the MVC did not exceed 2% over the entire period of the 12-week exercise intervention for the PF while for the DF leg, tendon strain values ranged between 5% and 6% (Figure 3). Furthermore, the average number of repetitions per training session over the 12 weeks of exercise intervention demonstrated a significant set effect ($F_{1.418,15.596}=29.515$, $p<0.001$) with no differences between legs (repetitions for DF vs. PF: 12 ± 2 vs. 12 ± 2 in Set 1, 10 ± 2 vs. 10 ± 1 in Set 2 and 9 ± 2 vs. 9 ± 1 in Set 3). This led to a factor of approximately 1.6 times higher integral of the joint moment over time for the DF in comparison with PF leg ($831\,610\pm210\,766$ Nm·s vs. $502\,632\pm169\,789$ Nm·s; $p<0.001$). Concerning TS activation volume analysis during training, no statistically significant differences were detected in the integral of the EMG activity over time during the cyclic contractions until failure between DF and PF independent of the analyzed muscle (GM, GL, and SO; Figure 2C).

3.2 | Muscle adaptive changes over time

There was a significant time effect on maximal plantarflexion joint moment ($F_{3.405,74.912}=48.931$, $p<0.001$)

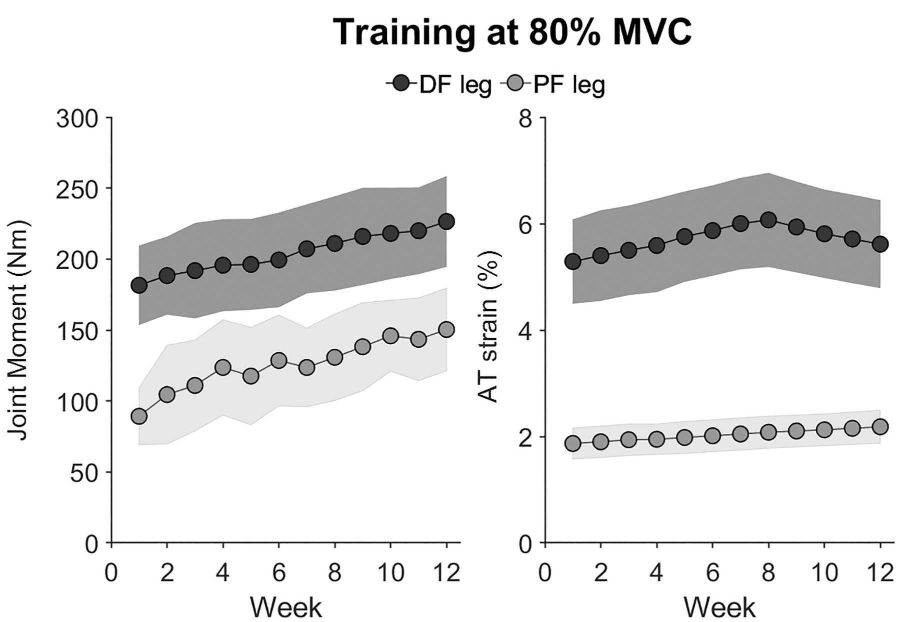

FIGURE 3 Weekly experienced ankle joint moment and Achilles tendon (AT) strain magnitudes during the exercise sessions of the 12 weeks of cyclic contractions until failure at 80% of the MVC in dorsiflexed (DF) and plantarflexed (PF) joint angle configuration (mean and SD values of the 12 examined subjects).

independent of the analyzed leg (no significant time × leg interaction). The post-hoc analyses revealed significantly higher maximal plantarflexion joint moments from W02 to W12 in comparison with PRE ($p < 0.001$) with an overall average relative increment of about 20% and 25% for DF and PF, respectively (Table 1; Figure 4). Following the initial increase at W02 a further successive significant increase was determined until W06 ($p < 0.001$; Table 1; Figure 4). As for the muscle strength gains, there was a significant time effect on muscle thickness and pennation angle for all three TS compartments (muscle thickness: GL $F_{2.897,63.729} = 152.820$, $p < 0.001$, GM $F_{3.124,68.718} = 50.960$, $p < 0.001$, SO $F_{3.099,68.179} = 135.536$, $p < 0.001$; pennation angle: GL $F_{3.965,87.226} = 164.057$, $p < 0.001$, GM $F_{3.226,70.981} = 94.382$, $p < 0.001$, SO $F_{2.396,52.705} = 104.682$, $p < 0.001$) with no significant leg effect or time × leg interaction. The post-hoc correction demonstrated significantly higher SO, GM, and GL muscle thickness and pennation angle values ($p < 0.001$) initially at W04 or at W06 followed by successive significant increases during following biweekly measurements (p ranged from <0.001 to 0.030; Table 1; Figure 5).

3.3 | Tendon adaptive changes over time

There was a significant time × leg interaction in maximal AT strain during MVC ($F_{2.115,46.525} = 22.204$, $p < 0.001$). The post-hoc analyses revealed successive significant increases ($p \leq 0.025$) in maximal AT strain until W08 after which there were successive significant decreases ($p < 0.001$) in the DF leg until the end of the 12-week intervention, coming closer to baseline tendon strain values (Table 1; Figure 4). On the contrary, the PF leg showed continuous successive significant increases ($p \leq 0.002$) until W10 (Table 1, Figure 4). At the end of the intervention the PF leg showed significantly higher AT strain values in comparison with the DF ($p = 0.040$) even though baseline values were not different between legs (Table 1, Figure 4). Concerning tendon stiffness, there was a significant time × leg interaction ($F_{4.372,96.189} = 3.266$, $p = 0.012$), with the post-hoc analyses showing significantly higher values at W12 in comparison with PRE for the DF leg ($p < 0.001$) without any changes for the PF over the period of the 12 weeks of loading exercise (Table 1). As for the tendon stiffness and maximal tendon strain, Young's modulus demonstrated a significant time × leg interaction ($F_{1.871,41.171} = 6.010$, $p = 0.006$) with the post-hoc analyses revealing greater values for the DF at W12 in comparison with PRE ($p = 0.037$; Table 1). The assessment of the free AT CSA at different lengths demonstrated a time × leg interaction ($F_{1.950,42.901} = 14.098$, $p < 0.001$). The post-hoc analyses revealed significantly higher values in comparison with PRE in all 10 intervals' CSA along the entire length of the free AT at W12 in the DF ($p \leq 0.023$) without any significant changes in the PF (Figure 6). Furthermore, the mean CSA of the free AT in the DF leg was at the end of the 12-week exercise intervention significantly greater in comparison with PRE and W08 ($p < 0.001$; Table 1). No significant main (leg or time point) effect nor time × leg interactions were detected neither for the free AT length nor for the peak spatial frequency analysis as a marker for the microstructural integrity of the proximal, mid, and distal AT (Table 1).

4 | DISCUSSION

The current study aimed to examine whether differences in the sensitivity to mechano-metabolic stimuli between muscle and tendon would lead to non-uniform adaptive changes in the TS MTU mechanical, morphological, and material properties. Two different lengths (DF vs. PF) of the MTU were implemented to vary the force potential of the muscle and hence the operating tendon strain and mechanical load during training between the two legs (DF: ~6% and ~204 Nm vs. PF: ~2% and ~126 Nm; Figure 3). Conversely, by performing each training set at 80% of the MVC and until failure, we aimed to induce a high but similar metabolic stress between the two legs as reflected by the similar EMG activity for all three TS compartments in DF and PF during the cyclic fatiguing contraction exercise (Figure 2C). Our hypothesis that tendon would be more sensitive to the operating magnitude of tendon strain but less to metabolic stress exercise was confirmed as tendon stiffness, Young's modulus and tendon CSA were only increased in the DF condition following the 12-week exercise intervention. In addition, the premise that skeletal muscle adapts at a higher rate than tendon and does not require high mechanical load to hypertrophy and increase its force potential during exercise was verified as the changes in morphological and mechanical properties of the muscle over time did not differ between DF and PF conditions.

Both legs demonstrated a similar adaptation rate and magnitude in muscle strength revealing an analogous overall increase of around 20% in the DF leg to 25% in the PF leg (Figure 4). In addition, a comparable adaptation rate and magnitude was also found in both muscle thickness and pennation angle of all three muscle compartments in DF and PF with overall increases of around 8%–13% (Figure 5). Given that the mechanical load during the 12 weeks of training was on average ~ 1.6 times higher in the DF compared with the PF leg, but induced a rather equal TS EMG activity between legs that led overall to a similar adaptation rate and magnitude in muscular

TABLE 1 Triceps surae muscle and tendon biomechanical properties for the leg trained in the dorsiflexed (DF) and in the plantarflexed (PF) joint angle configuration over the period of the 12 weeks of cyclic loading exercise until failure (mean and SD values of the 12 examined subjects).

	PRE	W02	W04	W06	W08	W10	W12
Ankle max moment (Nm)*							
DF	220 ± 41	233 ± 45[1]	244 ± 48[1–2]	251 ± 49[1–2]	259 ± 53[1–3]	259 ± 51[1–4]	264 ± 49[1–4]
PF	221 ± 31	236 ± 40[1]	247 ± 45[1–2]	257 ± 47[1–2]	262 ± 41[1–3]	275 ± 46[1–4]	277 ± 41[1–4]
AT max strain (%)§							
DF	5.4 ± 0.7	5.5 ± 0.9	5.7 ± 0.9[1–2]	6.0 ± 0.8[1–3]	6.3 ± 0.9[1–4]	6.0 ± 0.9[1–3,5]	5.8 ± 0.9[1–2,5–6,#]
PF	5.4 ± 0.8	5.5 ± 0.8	5.7 ± 0.9[1–2]	6.0 ± 0.9[1–3]	6.3 ± 1.0[1–4]	6.5 ± 0.9[1–5]	6.6 ± 0.9[1–5]
AT stiffness (Nmm^{-1})§							
DF	431 ± 91	446 ± 75	453 ± 90	444 ± 83	450 ± 97	456 ± 78	487 ± 97[1–6]
PF	409 ± 67	425 ± 55	432 ± 74	430 ± 74	416 ± 59	421 ± 57	426 ± 66
AT E-modulus (GPa)§							
DF	1.06 ± 0.19	–	–	–	1.08 ± 0.20	–	1.12 ± 0.19[1]
PF	1.05 ± 0.16	–	–	–	1.05 ± 0.13	–	1.01 ± 0.13
Mean AT CSA (mm^2)§							
DF	88.9 ± 11.6	–	–	–	90.6 ± 11.9	–	95.3 ± 13.4[1,5]
PF	89.0 ± 13.9	–	–	–	89.0 ± 14.9	–	89.4 ± 14.2
PSF at 25% AT (mm^{-1})							
DF	2.60 ± 0.16	–	–	–	2.56 ± 0.10	–	2.61 ± 0.12
PF	2.63 ± 0.18	–	–	–	2.54 ± 0.19	–	2.60 ± 0.17
PSF at 50% AT (mm^{-1})							
DF	2.66 ± 0.14	–	–	–	2.63 ± 0.11	–	2.70 ± 0.10
PF	2.63 ± 0.20	–	–	–	2.60 ± 0.12	–	2.61 ± 0.14
PSF at 75% AT (mm^{-1})							
DF	2.69 ± 0.16	–	–	–	2.60 ± 0.11	–	2.63 ± 0.13
PF	2.67 ± 0.19	–	–	–	2.61 ± 0.15	–	2.64 ± 0.13
Free AT length (mm)							
DF	55.5 ± 20.7	–	–	–	55.4 ± 20.7	–	55.7 ± 21.1
PF	59.1 ± 23.5	–	–	–	59.1 ± 23.5	–	59.1 ± 23.4
GL muscle thickness (mm)*							
DF	13.4 ± 1.5	13.4 ± 1.5	13.6 ± 1.5	13.9 ± 1.4[1–3]	14.3 ± 1.4[1–4]	14.5 ± 1.4[1–5]	14.9 ± 1.5[1–6]
PF	13.7 ± 1.7	13.7 ± 1.8	13.9 ± 1.7	14.3 ± 1.7[1–3]	14.6 ± 1.8[1–4]	14.8 ± 2.0[1–5]	15.1 ± 1.9[1–6]
GL pennation angle (°)*							
DF	14.4 ± 1.7	14.4 ± 1.6	14.6 ± 1.7	14.9 ± 1.7[1–3]	15.1 ± 1.7[1–4]	15.4 ± 1.8[1–5]	15.7 ± 1.8[1–6]
PF	13.8 ± 1.5	13.9 ± 1.5	14.1 ± 1.5	14.6 ± 1.6[1–3]	14.9 ± 1.7[1–4]	15.3 ± 1.8[1–5]	15.7 ± 1.8[1–6]
GM muscle thickness (mm)*							
DF	18.6 ± 2.3	18.6 ± 2.3	18.8 ± 2.1	19.3 ± 2.5[1–3]	19.6 ± 2.5[1–4]	20.0 ± 2.7[1–5]	20.3 ± 2.6[1–5]
PF	18.9 ± 2.6	19.0 ± 2.4	19.3 ± 2.6	19.6 ± 2.5[1–3]	20.0 ± 2.5[1–4]	20.4 ± 2.4[1–5]	20.6 ± 2.3[1–5]
GM pennation angle (°)*							
DF	21.7 ± 2.4	21.9 ± 2.4	21.9 ± 2.5	22.4 ± 2.2[1–3]	22.7 ± 2.4[1–4]	23.2 ± 2.5[1–5]	23.2 ± 2.3[1–5]
PF	21.7 ± 2.6	21.7 ± 2.6	22.0 ± 2.6	22.5 ± 2.7[1–3]	22.8 ± 2.5[1–4]	23.2 ± 2.7[1–5]	23.4 ± 2.7[1–5]
SO muscle thickness (mm)*							
DF	14.0 ± 2.4	14.1 ± 2.5	14.6 ± 2.5[1–2]	14.9 ± 2.3[1–3]	15.2 ± 2.4[1–4]	15.6 ± 2.4[1–5]	15.9 ± 2.5[1–6]
PF	14.1 ± 2.4	14.2 ± 2.4	14.6 ± 2.3[1–2]	15.0 ± 2.4[1–3]	15.2 ± 2.3[1–4]	15.6 ± 2.4[1–5]	15.8 ± 2.4[1–6]
SO pennation angle (°)*							
DF	19.6 ± 1.2	19.6 ± 1.2	20.1 ± 1.5[1–2]	20.4 ± 1.5[1–3]	21.0 ± 1.5[1–4]	21.3 ± 1.5[1–5]	21.5 ± 1.5[1–6]
PF	19.7 ± 1.5	19.8 ± 1.5	20.1 ± 1.4[1–2]	20.6 ± 1.5[1–3]	20.8 ± 1.5[1–4]	21.0 ± 1.5[1–5]	21.5 ± 1.4[1–6]

Abbreviations: AT, Achilles tendon; CSA, cross-sectional area; GL, m. gastrocnemius lateralis; GM, m. gastrocnemius medialis; PSF, peak spatial frequency; SO, m. soleus.

*Statistically significant time effect ($p < 0.05$). §Statistically significant time × leg interaction ($p < 0.05$). #Statistically significant differences to PF ($p < 0.05$).
[1]Statistically significant differences to PRE; [2,3,4,5,6] Statistically significant difference to time points W02, W04, W06, W08, and W10 ($p < 0.05$).

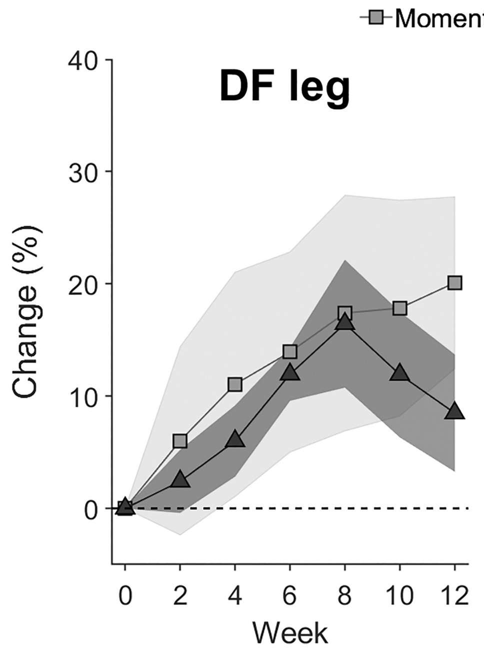

FIGURE 4 Relative (to PRE: Week 0) changes in maximal joint moment and maximal Achilles tendon (AT) strain during biweekly testing at 85° ankle joint angle of maximum voluntary isometric contractions (MVCs) for the dorsiflexed- (DF) and the plantarflexed- (PF) trained leg over the period of the 12 weeks of cyclic contractions until failure (mean and SD values of the 12 examined subjects).

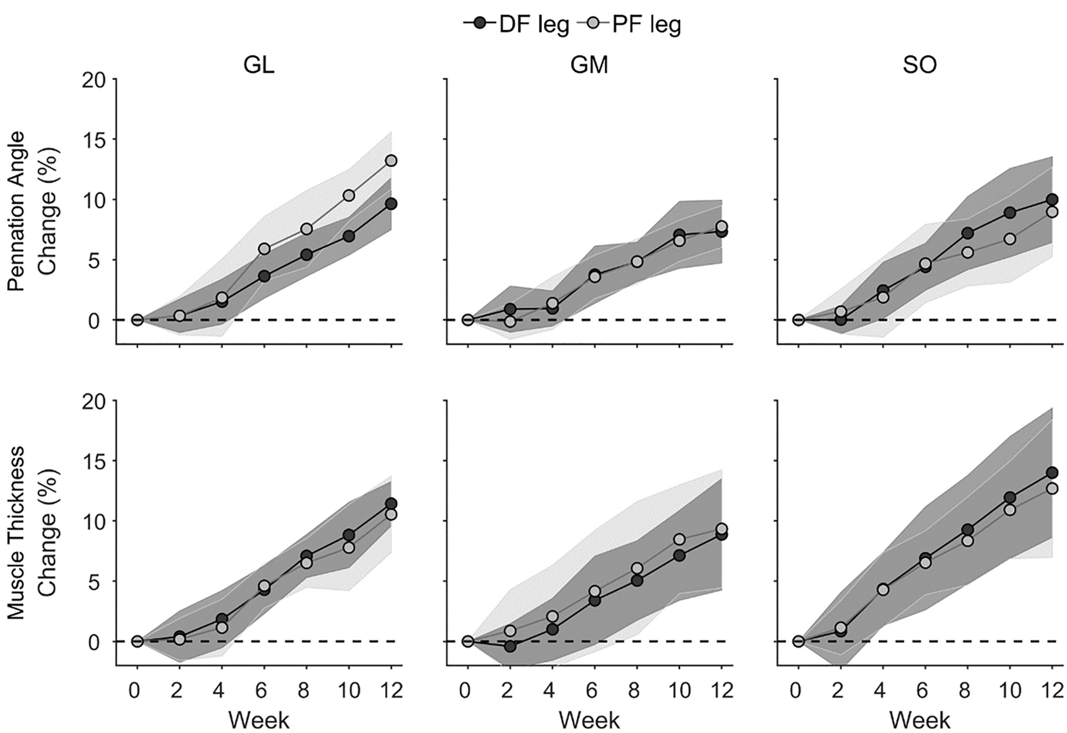

FIGURE 5 Relative (to PRE: Week 0) changes in pennation angle and muscle thickness of each triceps surae TS compartment (GM: m. gastrocnemius medialis; GL: m. gastrocnemius lateralis: SO: m. soleus) for the dorsiflexed (DF) and plantarflexed (PF) trained leg over the period of the 12 weeks of cyclic contractions until failure using biweekly recordings (mean and SD values of the 12 examined subjects).

properties, support the view that skeletal muscle is less sensitive to the experienced magnitude of mechanical load but more to muscle activation and metabolic stress during training. Moreover, the fact that the integral of the joint moment over time during training was also about 1.6 times higher for the DF compared with the PF condition,

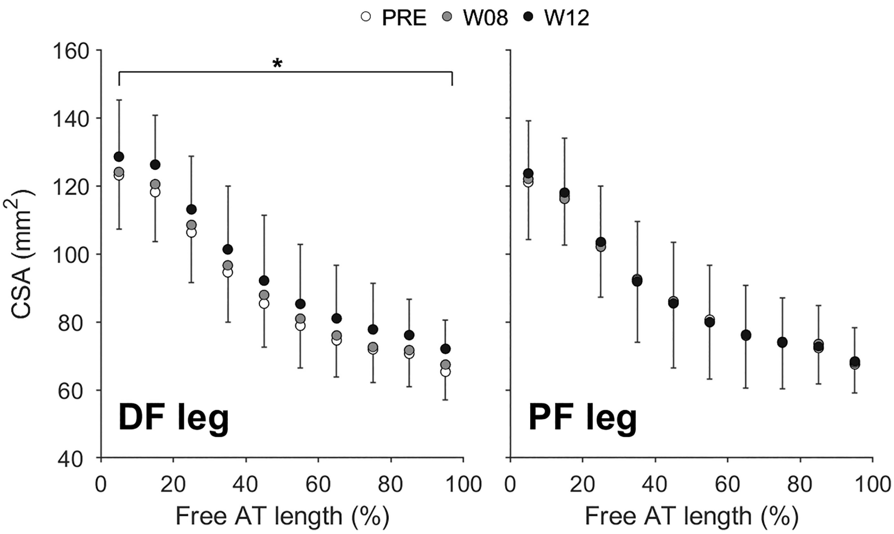

FIGURE 6 Free Achilles tendon (AT) cross-sectional area (CSA) in 10% intervals of the tendon before (PRE), post 8 (W08) and post 12 (W12) weeks of cyclic contractions until failure for the dorsiflexed (DF) and plantarflexed (PF) trained leg (mean and SD values of the 12 examined subjects). *: Statistically significant differences between PRE and W12 ($p < 0.05$).

further indicates that the volume of mechanical loading was not the primary stimulus triggering muscular adaptive changes.

In contrast to muscle, our data revealed clear differences in tendon adaptation between DF and PF leg. Tendon stiffness of the DF leg reached an overall increase of about 13% post 12 weeks of cyclic loading exercise while the PF leg demonstrated no significant increases throughout the intervention period (Table 1). The observed changes in tendon stiffness and related combined alterations in tendon's CSA and Young's modulus in the current study are in accordance with the range of adaptive increments reported in the literature using high-magnitude of mechanical loading exercises.[28] Similarly, the absence of adaptive changes in tendon stiffness in the PF leg can be explained due to the lack of alterations in AT size and material properties (Figure 6 and Table 1). Given that the operating tendon strain experienced during the 12 weeks of cyclic fatiguing contractions was on average ~6% and ~2% for the DF and PF legs, respectively, our data support the view that tendons are highly sensitive to the experienced magnitude of strain and that tendon strain values of clearly more than 2% are required to induce measurable adaptive changes in tendon biomechanical properties.[13,30] Moreover, our findings expand on previous knowledge showing that muscle and tendon adaptation to mechano-metabolic stimuli is different and that, in contrast to muscle, tendons are not sensitive to muscle metabolic stress exercise.

The consequence of the observed differences in muscle and tendon sensitivity in rate and magnitude to mechano-metabolic stimuli was a continuous (for the PF) or temporary (for the DF) maximal tendon strain increment during MVCs over the period of the 12-week intervention of exercising to muscular failure (Figure 4). Considering, that the ultimate tendon strain can be regarded rather constant[2] operating closer to its ultimate strain increases the risk of tissue damage and overuse injuries,[5] and therefore an increase in the operating tendon strain is proposed to indicate a higher mechanical demand for the tendon.[3] Therefore, it can be concluded that the tendon's lower adaptation rate to mechanical loading (in comparison with muscle) and lack of responsiveness to metabolic stress exercise can diminish its tolerance to high tensile loading during physical activity and disrupt its homeostasis.

Our conclusions concerning tendon adaptation are in line with previous investigations[13] showing that tendon strain values of 2.5% or less are not an appropriate stimulus to trigger adaptive changes within the tendon whereas high-strain magnitudes during exercise (over ~4.5%) are required to induce changes. A high-strain magnitude has been found through in vitro testing to correlate with greater tenocyte deformation, inhibition of a catabolic activity[31] and higher axial strain in collagen fibrils following crimp pattern disappearance in comparison with low strain levels, results which correspond well to in vivo studies. Our observed increases in AT stiffness in the DF leg by ~13% seem to be a combined effect of increased AT CSA (~6%) and Young's modulus (~7%). These findings are comparable to the literature but it needs to be emphasized that earlier studies demonstrated predominantly larger increases in Young's modulus in relation to AT hypertrophy.[13,28] Possible mechanisms behind the adaptive changes in AT biomechanical properties could be increased levels of collagen synthesis and expression as well as changes in collagen fibril morphology and upregulated cross-linking between collagen molecules.[12,32] Despite that, an improved structural integrity of tendinous tissue could be seen as a potential effect to an appropriate stimulus (e.g., high tendon strain), our in vivo investigation was unable to detect any exercise-related effects on peak spatial frequency over time (Table 1). This suggests that factors other than collagen packing density and alignment within tendon might be responsible for high-strain exercise-related changes in material and mechanical properties, or the chosen method is more sensitive toward larger-scale microstructural changes as seen with tendinopathic tendons.[33]

Concerning the muscle, the relative changes in mechanical and morphological properties in this study are in agreement with earlier studies analyzing TS adaptation to various mechanical and/or metabolic stress exercises (i.e., muscle strength increases of 21%–33%).[13,34] Training until failure[29] has been found to induce similar hypertrophy results as high-load training (70%–85% 1RM) and support the importance of metabolic stress on muscle adaptation. This may be explained by the greater phosphate inorganic accumulation leading to muscle failure (i.e., high metabolic stress) and ultimately to higher recruitment of fast-twitch fibers.[11] A further recently widely discussed exercise paradigm supposed to induce high metabolic stresses within the MTU during exercise is the use of vascular occlusion (blood flow restriction; BFR) during low-load muscular contractions. During such exercises, the ischemic condition in activated muscle leads to an accumulation of metabolites and ions, which can activate anabolic signaling pathways, increase growth hormone secretion and increase plasma levels of IGF-1, all that can promote muscle hypertrophy and strength gains even though the exercise limits the magnitude of mechanical load.[35] Despite the clear evidence in the literature that muscle responds well to metabolic stress and tendon to the experienced magnitude of tendon strain during exercise the results concerning the effects of BFR exercise on tendon are somehow contradictory in the literature. While Kubo et al.[36] have reported no significant effects of BFR on tendon properties, Centner et al.[37,38] have recently shown that both muscle and tendon respond well to low-load BFR training despite the low experienced mechanical loading and hence tendon strain magnitude. A clearer description of the mechanisms leading to potential tendon adaptations due to low-load BFR training and hence in the absence of appropriate experienced tendon strain magnitudes is required. Based on the current findings, we have no evidence to suggest that tendon effectively adapts to high metabolic stresses induced by muscular contractions until failure at low strain values.

In the current study, MTU measurements were taken biweekly to create a more complete profile of the temporal dynamics of muscle and tendon adaptation to mechano-metabolic stimuli. The timepoint of first significant muscle strength increase in both legs (W02 with an overall average increment of about 6%) as well as the slower rate of increment or plateau at the end of the 12-week training program are in line with previous investigations using high mechanical loading or metabolic stress exercises.[8] However, in contrast to muscle, there is limited information about the time course of human tendon adaptation to mechano-metabolic stimuli. To our knowledge, only Kubo et al.[34] analyzed mechanical load exercise-related adaptive changes in human AT properties over time. Using a monthly MTU testing procedure over a 3-month high magnitude resistance exercise period they reported adaptive increments in the AT stiffness post 3 months with no alterations at the second month. Given that our results agree with the study of Kubo et al.[34] further supports the notion that the temporal dynamics of muscle and tendon adaptation are different and that human tendon requires more than 8 weeks of high-strain loading exercise to cause adaptive changes in the tendon's mechanical properties. This is further supported by the fact that neither morphological nor material alterations were determined at W08, even if assuring more beneficial conditions (higher mechanical loading in DF leg) for an effective tendon adaptation. This inhomogeneous adaptation rate between muscle and tendon led to muscle strength gains in the absence of any changes in tendon's mechanical properties (i.e., tendon stiffness) and hence to a corresponding increase in maximal tendon strain, which indicates to a higher mechanical demand for the tendon.[3] Since tendon strain is well accepted as a relevant determinant for tendon failure during mechanical loading[4] and a mechanical risk marker for the initiation and progression of tendon injuries,[39] such differences in the temporal dynamics of muscle and tendon adaptation to mechanical loading need to be considered in practical and therapeutic settings in order to reduce the risk of tendon overloading during exercise.

It is important to note that performing the isometric plantarflexion contractions at a flexed knee joint angle places the biarticular gastrocnemii muscles at a less favorable condition to produce force[22] and that the soleus muscle may contribute more to the resultant ankle joint moment. This might be in particular the case at the PF position due to further muscle shortening potentially reaching the region of active insufficiency for the gastrocnemii. This is supported by the observation that while the peak joint moments during 80% MVC training at PF increased by a factor 1.6, the operating tendon strain measured at the GM MTJ remained constantly around 2% over the period of the 12-week intervention (see Figure 3). However, the rate and magnitude of adaptive changes in muscle thickness and pennation angle were similar between the gastrocnemii and soleus as well as between DF and PF legs (Figure 5) supporting our conclusion that the primary stimulus for muscle hypertrophy is not the magnitude of mechanical loading but is rather related to muscle activation and metabolic stress.

One might argue that co-activation from antagonistic or synergistic muscles was not accounted for, which could in absolute terms affect our estimated AT force and correspondingly the AT stiffness and Young's modulus. However, the antagonistic moment contribution to the generated plantarflexion moment during maximal isometric contractions seems rather low in healthy young adults.[40] Hence, this would have a negligible influence on the outcomes especially in relative terms due to the intra-subject protocol used in this study and the implementation of the same joint configurations throughout our MTU testing. More importantly, the main observation that the maximal tendon strain increased more in the PF over time in relation to the DF leg is not affected by these limitations. Finally, even though we implemented a training protocol of muscular contractions until failure in both DF and PF conditions at a similar relative force level (80% of the corresponding MVCs) and we have shown that muscle activity over time estimated via EMG recordings as well as the average number of repetitions to reach volitional muscular failure for each set were not different between legs, the magnitude of metabolic stress within the muscle is not known and may also be difficult to control accurately in vivo. Moreover, whether muscular contractions until failure induce similar levels of metabolic stresses within the tendon is difficult to address. However, the fact that the magnitude and volume of mechanical loading (generated joint moment) during training was ~1.6 times higher in the DF compared with PF leg and that the operating tendon strain during training were on average ~6% and ~2% respectively, provides clear evidence that muscular contractions until failure at low force potential due to the force–length–velocity relationship cannot be recommended as an effective stimulus to induce adaptive changes in tendon mechanical, material, or morphological properties.

In conclusion, the present study provides evidence that the responsiveness of muscle and tendon to mechano-metabolic stimuli differs in rate and magnitude. Moreover, muscle has been shown to be highly sensitive to muscle activity and metabolic stress irrespective of loading level, whereas tendon adaptation depends primarily on the magnitude of experienced strain and mechanical load. Such differences between muscle and tendon sensitivity to mechano-metabolic stimuli may temporarily cause an imbalance in the adaptation within the MTU that could have implications for the risk of tendon overuse injury.

5 | PERSPECTIVES

Muscle and tendon adapt differently to mechano-metabolic stimuli. In the current study, we trained both legs with fixed-end plantarflexion contractions until failure using high muscle activity to induce high metabolic stress but at different muscle–tendon unit lengths to vary the muscle force potential and hence the experienced tendon strain during training. Only the leg trained under a higher mechanical load demonstrated adaptive changes in tendon mechanical and morphological properties while muscle strength gains and muscle hypertrophy were similar between legs post-exercise. Training at lower operating tendon strains (~2%) led to continuous increments in maximal tendon strain, whereas higher experienced tendon strains (~6%) caused only temporary increases before returning close to baseline values. Given that a higher experienced tendon stain can initiate microdamages we argue that if training occurs with low tendon strains (e.g., via muscle fatiguing exercises or low force potential due to the force–length–velocity relationship), tendon adaptations may not keep up with muscle adaptations, which could potentially increase the risk for tendon overuse injuries. Sports and medical practitioners focusing on muscle strength gains need to consider this and should explore whether interventions based on experienced tendon strain rather than the level of muscle demand could help to minimize muscle–tendon imbalances.

AUTHOR CONTRIBUTIONS

YL, GE, AA, and KK: Conceived and designed the experiments. YL and GE: Performed the experiments, analyzed the data, and prepared the figures. YL, GE, AA, and KK: Interpreted the results and drafted the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Finally, all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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CONFLICT OF INTEREST STATEMENT

KK has equity in Protendon GmbH & Co. KG, whose measurement device and software were used for the data processing and analysis in this study. No competing interests are declared by the remaining authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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