MUSCLE AND TENDON PLASTICITY AND INTERACTION IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

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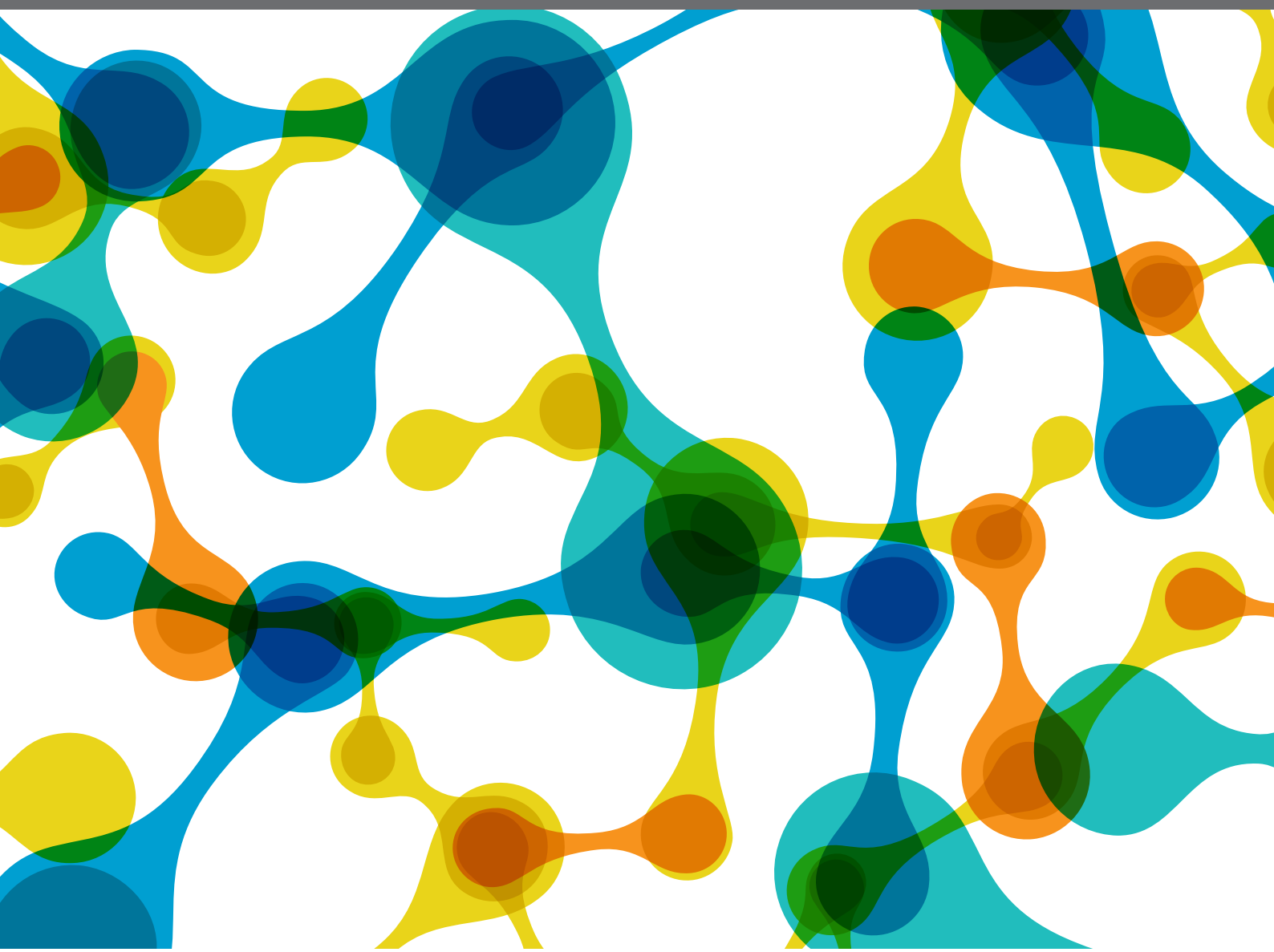

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MUSCLE AND TENDON PLASTICITY AND INTERACTION IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

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EDITORIAL
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Editorial: Muscle and Tendon Plasticity and Interaction in Physiological and Pathological Conditions

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Keywords: adaptation, muscle contraction, strain energy, loading, maturation, aging, disease, injury risk

Editorial on the Research Topic

Muscle and Tendon Plasticity and Interaction in Physiological and Pathological Conditions

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The interaction of muscle and tendon during movement is a crucial element for the performance of musculoskeletal systems. As both muscle and tendon are mechanosensitive and adapt to their mechanical environment, mechanical loading can introduce cellular responses affecting the properties of these tissues and the functional interplay between them. However, aging and pathology affect the muscle-tendon unit (MTU) in various ways, with direct consequences on its functional capacities. This Research Topic of Frontiers in Physiology focuses on current research considering muscle and tendon plasticity and their interaction during movement in physiological and pathological conditions.

It has been long recognized that the energy exchange within the MTU affects the operating conditions of muscle fibers and, thus, human performance during running and jumping. In their contribution, Hollville et al. demonstrated that the fascicle behavior of the vastus lateralis and gastrocnemius medialis muscle during countermovement jumping is not affected by the compliance of common sport surfaces (i.e., artificial turf, hybrid turf, and athletic track), while they recognized the possible role of the nervous systems to regulate muscular behavior to compensate for the differences in surface properties. Sano et al. showed for the first time that the interaction of muscle and tendon is also relevant during human swimming. As for terrestrial locomotion, the tendinous tissue of the quadriceps muscles seems to uncouple muscle and MTU strain, store and release energy and, thus, decrease the fascicle motion of the vastus lateralis muscle during a dolphin-kick. Moreover, the authors observed an increasing contribution of the tendinous tissue to MTU lengthening and shortening at higher swimming speed. Changes in tendon lengthening are predominantly governed by the magnitude of muscle force exertion, as suggested by the findings of Rosario and Roberts from a rat model, where loading rate had little influence on tendon fascicle mechanics. Despite tendon viscoelasticity, effects of loading rate on muscle fascicle behavior may only be relevant in MTUs with extremely high tendon-to-fiber length ratios, as observed in certain animals. Other contributions to this Research Topic also looked at intramuscular mechanisms that affect the operating conditions of its fibers. Konow et al. demonstrated that the shape-change of the rat gastrocnemius medialis muscle during contractions is tuned

to the task requirements. Muscle bulging in the width direction was predominantly observed at high forces and may favor whole muscle force generation, while the greater bulging in thickness at low forces may facilitate muscle shortening. The understanding of muscle and tendon mechanics also progresses in research on injury and disease. In this article collection, Agres et al. showed that lengthening of gastrocnemius medialis fascicles is limited during passive ankle dorsiflexions, following Achilles tendon rupture, probably due to a longer and more compliant tendon. Moreover, this altered behavior does not seem to change with measures that primarily focus on the rehabilitation of independent gait. Tendons are also affected following muscle injury, as Barin et al. showed in a rat model, providing evidence of substantial extracellular matrix remodeling and changes in mechanical properties of the calcaneal tendon. Some of our contributors also examined the effects of pathologies affecting MTU function. Hösl et al. reported that children with cerebral palsy have lower gastrocnemius medialis muscle thickness and shorter fascicle compared with healthy peers, which directly associated with walking speed. During walking, the fascicles operated at shorter length, even normalized to resting length, and with lower velocities. Potential approaches for therapy are discussed by Kalkman et al. Their comprehensive review elaborated why stretching does not improve muscle morphology and function in children with cerebral palsy, and discusses alternative approaches such as combined therapies, eccentric training, or interventions for increasing tendon stiffness.

Across the human lifespan, both muscle and tendon adapt to changes of their mechanical environment. However, the degree of tissue plasticity may change with maturation and aging and muscles and tendons may differ considering their responsiveness to certain types of loading. Pentidis et al. reported that preadolescent artistic gymnastic athletes show higher muscle strength when normalized to body mass and jump performance, in spite of similar Achilles tendon stiffness compared to controls. In early and late adolescents, Charcharis et al. observed greater quadriceps femoris muscle strength and patellar tendon stiffness compared to untrained peers, although higher tendon strains were more prevalent in trained adolescents, which indicates an increased mechanical demand for the tendon. The data further suggests an increased risk for imbalances of muscle strength and tendon stiffness until adulthood. Mersmann et al. showed that in adolescent basketball athletes, high levels of tendon strain are associated with impairments of proximal tendon micromorphology similar to those observed with tendinopathy. In adult middle-distance runners, Devaprakash et al. found both higher plantar flexor muscle strength and Achilles tendon stiffness compared to untrained controls. The authors concluded that the shorter and stiffer tendons may favor rapid force production and reduce the risk of tendon fatigue injury, yet longer MRI T2* relaxation times of the free Achilles tendon could indicate accumulated damage. In their cross-sectional observations, Epro et al. showed that plantar flexor muscle strength and Achilles tendon stiffness are greater in the dominant leg of adult elite track and field jumpers, yet in a similar order of magnitude, suggesting a balanced adaptation of muscle and tendon. However, the same authors (Karamanidis and Epro) also presented evidence of imbalances when the adaptation of muscle and tendon was monitored over time. In a 1-year period of observation with measurements performed every 3–5 weeks, track and field jumpers had greater fluctuations of maximum tendon strain compared to untrained controls. Arampatzis et al. argued in their perspective paper that well-balanced muscle strength and tendon stiffness are important for healthy and effective MTU function and present a framework how it could be promoted by individualized muscle and tendon diagnostics and load prescriptions. Tailored loading for the tendon may also be a future approach in the treatment of tendinopathy. Wiesinger et al. provided evidence that tendon stiffness is reduced in patients with chronic patellar tendinopathy, while no significant differences were shown considering the capacity to store and release energy. Although the restoration of tendon mechanical properties may be targeted or monitored in therapeutic interventions, Kulig et al. called in their perspective article for a multilevel perspective in the treatment of tendinosis, highlighted the importance of the etiology of this condition and emphasized the necessity to also address the changes observed in the motor control system.

Considering muscle adaptation, Geremia et al. investigated changes in triceps surae muscle architecture in response to 12 weeks of eccentric training. All three muscles demonstrated an increase in muscle thickness and fascicle length, with the greatest changes of fascicle length in the soleus muscle. Moreover, Marzilger et al. showed on the vastus lateralis that, when time under tension and load magnitude is matched, the lengthening velocity during eccentric loading does not affect the extent of muscle hypertrophy. McMahon et al. shed light into the role of tumor necrosis factor alpha (TNFα) in the regulation of muscle mass during training and detraining. While in response to 8 weeks of resistance training no changes in systemic levels of TNFα were observed, the reduction of muscle mass during the following detraining period seemed to be mediated by the pro-inflammatory cytokine in healthy adults. In their review, Csapo et al. focused on the extracellular matrix of skeletal muscle, providing an overview about its composition, function, remodeling and adaptation. The authors concluded that its essential role is widely underestimated and may further be a target in the treatment of muscular and metabolic disorders consequent to aging or disease. The aging muscle was also subject of the original research article by Bruseghini et al. demonstrating that both aerobic high-intensity training and isoinertial resistance training reduce intermuscular adipose tissue and increase muscle volume of the quadriceps, yet only resistance training increases muscle physiological cross-sectional area, activation and strength in elderly men. The simplified method of muscle volume prediction for the triceps surae presented by Karamanidis et al. may be of use in future studies on the elderly and further provides evidence that age-induced atrophy may, in addition to its dimensions, change the muscle shape, at least in women as observed here. Singh et al. investigated the effects of a combined aerobic and resistance training, performed either one, two or three times a week, on walking economy of postmenopausal women. Regardless of training frequency, it seems that 16 weeks of combined training improves the ease

of walking and economy. Finally, Venturelli et al. presented a case report of a young patient suggesting that resistance training can improve muscle strength and respiratory capacity in mitochondrial encephalomyopathy, lactic acidosis, and recurrent stroke-like episodes syndrome, a rare degenerative disease that is associated with massive deconditioning.

Our understanding on how muscle and tendon interact and how the MTU adapts to changes in mechanical loading as well as the influence of maturation, aging and pathology is continuously increasing. The present article collection sampled exciting new developments in these areas, yet it also points at many unanswered and new questions in this field. Those include the question of the optimization of muscle-tendon interaction via mechanical loading for increasing, maintaining or regaining movement performance. A better grasp of the complexity of three-dimensional muscle and tendon architecture remains one of the challenges to further research on MTU function. Likewise, more insight into the role and malleability of the extracellular matrix components is still critically missing. The processes of mechanotransduction in muscle and tendon are not well-understood, especially when it comes to potential changes due to aging or pathology. Advances in this area may contribute to increasing the effectiveness and efficiency of loading-based interventions and improve treatment success e.g., in tendinopathy or cerebral palsy. We would like to thank all contributors for their participation to this Research Topic and look forward to join our efforts in muscle and tendon research.

AUTHOR CONTRIBUTIONS

This manuscript was written by all authors. All authors have performed editorial reviewing tasks for this Research Topic.

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CASE REPORT
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Neuromuscular and Muscle Metabolic Functions in MELAS Before and After Resistance Training: A Case Study

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Mitochondrial encephalomyopathy, lactic acidosis, and recurrent stroke-like episodes syndrome (MELAS) is a rare degenerative disease. Recent studies have shown that resistant training (RT) can ameliorate muscular force in mitochondrial diseases. However, the effects of RT in MELAS are unknown. The aim of this case report was to investigate the effects of RT on skeletal muscle and mitochondrial function in a 21-years old patient with MELAS. RT included 12 weeks of RT at 85% of 1 repetition maximum. Body composition (DXA), *in vivo* mitochondrial respiration capacity (mVO_2) utilizing Near-infrared spectroscopy on the right plantar-flexor muscles, maximal voluntary torque (MVC), electrically evoked resting twitch (EET) and maximal voluntary activation (VMA) of the right leg extensors (LE) muscles were measured with the interpolated twitch technique. The participant with MELAS exhibited a marked increase in body mass (1.4 kg) and thigh muscle mass (0.3 kg). After the training period MVC (+5.5 Nm), EET (+2.1 N·m) and VMA (+13.1%) were ameliorated. Data of mVO_2 revealed negligible changes in the end-exercise mVO_2 (0.02 mM min^{-1}), Δ mVO_2 (0.09 mM min^{-1}), while there was a marked amelioration in the kinetics of mVO_2 (τ mVO_2; Δ70.2 s). This is the first report of RT-induced ameliorations on skeletal muscle and mitochondrial function in MELAS. This case study suggests a preserved plasticity in the skeletal muscle of a patient with MELAS. RT appears to be an effective method to increase skeletal muscle function, and this effect is mediated by both neuromuscular and mitochondrial adaptations.

Keywords: MELAS, exercise, neuromuscular function, muscle respiratory capacity, resistance training

INTRODUCTION

Mitochondrial diseases caused by mitochondrial DNA mutations are rare pathologies causing devastating physical and neural impairments (Matsumoto et al., 2005; Sproule and Kaufmann, 2008). Among these pathologies, mitochondrial encephalomyopathy, lactic acidosis, and recurrent stroke-like episodes syndrome (MELAS) is a rare neurodegenerative disease affecting several

organs, particularly the nervous system and skeletal muscles with a population prevalence of 236/100000 (Matsumoto et al., 2005). The symptoms of MELAS include muscle weakness, recurrent headaches, loss of appetite, and seizures. Stroke-like episodes are common, often precipitating muscle weakness, unconsciousness, vision abnormalities and brain damage, mobility impairment, and a loss of cognitive function (Thambisetty and Newman, 2004; Henry et al., 2017). Patients with MELAS generally have a poor prognosis, as effective therapies for MELAS have yet to be found (Pfeffer et al., 2012).

Given the central role of mitochondria in energy metabolism, patients with mitochondrial dysfunctions have severe exercise intolerance (Tarnopolsky and Raha, 2005) and patients are often advised to avoid exercise, which leads to a vicious-cycle of deconditioning. Contrasting with these recommendations, recent studies have shown that aerobic exercise is actually feasible without adverse events, and even beneficial, with the prospect that this type of intervention may prevent physical deconditioning, and attenuate exercise intolerance and fatigability in these patients (Taivassalo et al., 1996, 1998, 1999; Siciliano et al., 2000). Other important achievements of the exercise training in this population are the ameliorations of whole-body aerobic capacity and muscle oxidative metabolism (Taivassalo et al., 2001; Cejudo et al., 2005; Taivassalo and Haller, 2005; Jeppesen et al., 2006). It is important to note, that besides a remarkable impairment of the aerobic metabolism, patients with MELAS also presents severe skeletal muscle losses. This imply that, perhaps, a training method that can induce both positive changes of mitochondrial (Porter et al., 2015) and neuromuscular function of skeletal muscle may provide large benefits in terms of exercise capacity and quality of life. However, to date, the research on this matter in patient with MELAS has mainly focused on the effects of very light aerobic exercise (Sproule and Kaufmann, 2008), and limited data are available on other training approaches.

Yet, some studies have reported specific positive effects of resistant training (RT) in patients with neurological and skeletal muscle dysfunctions (Tollback et al., 1999; Voet et al., 2013). Noticeably, the studies of Taivassalo et al., 1998, 1999, 2006 and Taivassalo and Haller (2004, 2005); and the recent study of Murphy et al. (2008) reported a clear physiological rationale for the utilization of RT in patients with mitochondrial dysfunction. In this last promising study have been reported improvements of muscle strength (15–25%), and ameliorations of oxidative capacity, reflected by an increase of oxygen extraction and changes in the percentage of COX deficient (Murphy et al., 2008). However, the effects of RT on the neuromuscular function and mitochondrial oxidative capacity in MELAS are currently unknown.

This study sought to investigate the effects of RT on skeletal muscle and mitochondrial function in a 21 years old male patient with MELAS. Specifically, by studying the muscles mass, neuromuscular function of locomotor-limb and *in vivo* muscular respiratory capacity after 12 weeks of RT we tested the following hypotheses: (1) After the training period, the locomotor-limb function would be increased, (2) this amelioration in muscle function could be explained, perhaps at least in part, by the improvement of neuromuscular and structural factors, and (3) *in vivo* muscle respiratory capacity would be improved in the skeletal muscle tissue recruited during the RT.

CASE PRESENTATION

Participant

The participant was a 21-years-old male patient with MELAS characterized by *cytochrome c oxidase* dysfunction. At the time of study, the participant suffered of severe mobility impairment (wheelchair limited), loss of hearing, partial blindness and dysphagia. Muscle weakness and asthenia were coupled with an exacerbated fatigue. From a cardiovascular point of view the heart function was reduced leading to the necessity to implant a DDDR pacemaker. The participant's clinical characteristics were determined by qualified medical members of the research team (**Table 1**). Before testing, the participant abstained from physical rehabilitation for 48 h, caffeine for 12 h, and food for 3 h, and was not taking any drugs known to impact the response to the assessment procedures. This study was carried out in accordance with the recommendations of the Declaration of Helsinki. The protocol was approved by the Department of Neuroscience Biomedicine and Movement Science (Prot 227). Caregiver of the case gave written informed consent for the case participation in the study and publication of this case report. In order to better categorize the singular data of the participants with MELAS, a group of eight healthy age- sex-matched subjects served as control group (CTRL). All CTRL subjects gave written informed consent. CTRL subjects were healthy recreationally active men, demographic characteristics are reported in **Table 1**.

Experimental Overview

The participants visited the laboratory on three occasions separated by 24 h. The first visit comprised body composition

TABLE 1 | Subject characteristics.

	MELAS		CTRL
	PRE	POST	
Age (years)	21	21	22 ± 2
Digit Span	3	3	
Memory of Prose (Range 0–28)	Memory 4 Recall 8	Memory 4 Recall 8	
Rey Auditory Verbal Learning Test (Range 0–75)	Memory 23/75 Recall 6/15	Memory 23/75 Recall 6/15	
Clock Drawing Test (Range 0–10)	0	0	
Frontal Assessment Battery (Range 0–18)	8	8	

Data of the control participants (CTRL) are given as mean ± standard deviation. MELAS, Mitochondrial encephalomyopathy, lactic acidosis, and recurrent stroke-like episodes syndrome.

testing (DXA), and the clinical assessments. At the second visit, the participants completed a familiarization with the interpolated twitch and *in vivo* mitochondrial respiration capacity protocols. At the third visit, the participants completed an *in vivo* mitochondrial respiration capacity protocol utilizing a Near-infrared spectroscopy device (NIRS) on the right plantar-flexor muscles. After 60 min of recovery, maximal voluntary torque (MVC), electrically evoked resting twitch (EET) and maximal voluntary activation (VMA) of the right leg extensors (LE) muscles were determiner with the interpolated twitch technique. Only for the participant with MELAS these evaluations were repeated after 12 weeks of RT.

Clinical Assessments

From a clinical point of view, the participant with MELAS showed dysphagia to solids (he had a modified diet and assumed food integrators). He presented with easy fatigability and decreased muscle mass. No limitation of (main joints) range of motion was found at the upper and lower limbs. Muscle tone was not affected in a relevant way but needed some assistance to change posture (from supine to sitting posture as well as from sitting to standing posture).

The cognitive assessment battery utilized in this study was very limited due to the severe hearing loss, vision acuity deficits and hemianopia, in addition to the easy fatigue and eyelid ptosis. The evaluation was focused on the main cognitive deficits reported in the literature in the MELAS. The patient was perfectly oriented in the space-time parameters, but with important deficits of sustained and selective attention, with an easy distractibility. The participant demonstrated deficits in short-term and long-term verbal memory. The evaluation of executive functions estimated with Frontal Assessment Battery and Clock Drawing Test (Appollonio et al., 2005) revealed a lack of planning ability, verbal ideation and inhibitory control. Tests' scoring are reported in **Table 1**.

Body Composition

Body composition (body fat and lean mass) was assessed by means of Dual energy X-ray absorptiometry using a total body scanner (QDR Explorer W, Hologic, MA, United States; fan-bean technology, software for Windows XP version 12.6.1) according to the manufacturer's procedures. The scanner was calibrated daily against the standard supplied by the manufacturer to avoid possible baseline drift. Whole body scanning time was about 7 min. Data were analyzed using standard body region markers: upper and lower extremities, head, and trunk (pelvic triangle plus chest or abdomen). Additionally, the DXA scans were examined using non-standard body region markers to define thigh segments. The thigh region was delineated by an upper border formed by an oblique line passing through the femoral neck to the horizontal line passing through the knee (Skalsky et al., 2009). All scanning and analyses were performed by the same operator to ensure consistency. In our lab the precision error (percent coefficient of variation with repositioning) of whole-body DXA measurements is 2.3, 0.5, and 2.8% for fat mass, lean mass and percent fat mass, respectively.

In vivo Mitochondrial Respiration Capacity

The assessment of *in vivo* mitochondrial respiration capacity was performed via a non-invasive approach of the muscle oxygen consumption (mVO_2) as previously described by Ryan et al. (2013, 2014a), Adami and Rossiter (2018). NIRS data were obtained using a device (OxiplexTS, ISS, Champaign, IL, United States), equipped with a standard acquisition probe (emitter detector distances of 2.0, 2.5, 3.0, and 3.5 cm). The values of oxygenated hemoglobin (Gonzalez-Alonso et al., 2001) and deoxygenated hemoglobin [HHb] were recorded at 4 Hz and expressed in micromoles using the Beer Lambert Law and multi-distance frequency resolved spectroscopy.

The NIRS probe was positioned longitudinally on the belly of the right plantar flexor muscles. The probe was secured with double-sided adhesive tape and a Velcro strap around the calf. After 30 min of warm-up period, the NIRS device was calibrated using a phantom with known optical properties A blood pressure cuff was placed proximal to the NIRS probe around the popliteal area. The blood pressure cuff was controlled with a rapid-inflation system (Hokanson E20, D.E. Hokanson) set to a pressure of >250 mmHg and powered with an air compressor.

The NIRS experimental protocol consisted of two measurements of resting mVO_2 after the inflation of the blood pressure cuff for 30 s. mVO_2 was calculated as the rate of change of the HHb signal during the arterial occlusion via linear regression. Following the resting measurements, participants performed a 30 s dynamic contractions of the plantar flexors muscle to increase mVO_2. Upon relaxation, the recovery kinetics of mVO_2 were measured using a series of transient arterial occlusions with the following timing: 5 s on/5 s off for occlusions 1–5, 7 s on/7 s off for occlusions 6–10, and 10 s on/10 s off for occlusions 11–20. Post-exercise mVO_2 was calculated for each occlusion using a linear regression. The mVO_2 recovery kinetics were determined by fitting the time-dependent changes during the recovery period to a mono-exponential curve described by the following equation:

$$Y(t) = Y_{end} + Y_{amp}(1 - e^{-(t-TD/\tau)}) \quad (1)$$

where Y_{end} is the level of variable measured at end-of-exercise and Y_{res} refers to the amplitude of the response, TD represent the time delay (TD), and τ reflects the time constant of the recovery, a relative measure of muscle oxidative capacity (Adami and Rossiter, 2018). Model variables were determined with an iterative process by minimizing the sum of squared residuals (RSS) between the fitted function and the observed values. Goodness of fit was assessed by visual inspection of the residual plot and the frequency plot distribution of the residuals, Chi square values, and the coefficient of determination (r^2), which was calculated as follows:

$$r^2 = 1 - (SS_{reg}/SS_{tot}) \quad (2)$$

with SS_{reg}, the sum of squares of the residuals from the fit and SS_{tot}, and the sum of squares of the residuals from the mean.

Neuromuscular Function of Locomotor-Limb

Maximal voluntary and electrically evoked muscle contractions of the LE muscles were measured utilizing a custom-made setup (Venturelli et al., 2015). Subjects were seated in an upright position with back support. The hip and the knee were flexed at 90°, and the right ankle were attached, via a strap and rigid steel bar, to a force transducer (DBBSE-100 kg, A2829. Applied Measurements Limited, Aldermaston Berkshire, United Kingdom). The output from the force transducer was amplified (INT2-L, London Electronics Limited, Sandy Bedfordshire, United Kingdom), and recorded at a sampling rate of 5 KHz with a PowerLab-16/35 data acquisition system (ADInstruments, Bella Vista, NSW, Australia).

Voluntary and EET Normalized Force

In the participant with MELAS the determination of muscle cross sectional area via magnetic resonance imaging was not possible due to the implanted pacemaker. Therefore, LE voluntary muscle normalized force was calculated by dividing torque of the LE isometric maximal voluntary contraction (MVC), by the lean muscle mass of the corresponding muscles (nMVC) from DXA. Similarly, EET normalized force was calculated by dividing torque of the LE electrically evoked EET, by the lean muscle mass of the corresponding muscle (nEET).

$$\text{VoluntaryandEETnormalizedforce} = \text{torque/thighleanmusclemass} \quad (3)$$

Electromyography

M-waves were recorded during femoral nerve stimulation in the vastus lateralis muscle (detailed in next section). Pairs of full-surface solid adhesive hydrogel electrodes (H59P, Tyco Healthcare Group, Mansfield, MA, United States) were positioned lengthwise over the muscle belly, with an inter-electrode distance (center-to-center) of 20 mm. The ground electrodes were fixed over the ipsilateral patella. Light skin abrasion followed by skin cleansing kept electrical impedance below 10 kΩ. EMG signals were amplified with a pass-band of 10 Hz–1 kHz and digitized online at a sampling frequency of 5 kHz.

Nerve Stimulation

Each test procedure began with the determination of the maximal M-wave and EET responses in the resting LE muscle. Briefly, current intensity was progressively increased from 0 mA to the value beyond which there was no further increase in M-wave amplitude. The stimulus utilized for the study was set at the 125% of the intensity required to produce a maximal M-wave response. Electrical stimuli were delivered using circular (diameter 5.0 cm) self-adhesive electrodes (Dermatrode, American Imex, Irvine, CA, United States) positioned in the femoral triangle, 3–5 cm below the inguinal ligament, and the anode placed over the iliac crest. The EET were evoked in the passive muscle using electrical stimulation consisting of single square-wave pulses of 0.1-ms duration, delivered by a Digitimer DS7h constant-current stimulator (Digitimer Ltd., Welwyn Garden City, United Kingdom). The EET was measured 5 s after a 5 s MVC of the LE and this procedure was repeated six times. Consequently, EET was assessed in the potentiated state. The interval between the MVCs was 30 s. Peak torque, was assessed for each EET (Sandiford et al., 2005). Voluntary activation of the LE muscles during the MVCs was assessed using a superimposed twitch technique (Merton, 1954). Briefly, the force produced during a single twitch superimposed on the MVC was compared with the force produced by the electrically evoked EET produced, at rest, 5 s after the MVC.

Exercise Resistance Training

Resistant training included 60 min three times a week of high-intensity strength training for an overall exercise duration of 12 weeks. Sessions started with 10 min of warm up which included active joint mobilization of lower and upper limbs. Then, the participant performed 3 sets of 10 reps of strength exercises at 85% of 1 repetition maximum (1RM). 1RM was determined by means of Brzycki method. Briefly, the participant executed progressive series of the isotonic exercise until the offered resistance was impossible to be sustained for 5/6 repetitions. The number of repetitions, and the relative workload, were used in the Brzycki equation. 1RM was adjusted every 2 weeks and the corresponded training load was increased. RT ended with stretching exercises for all the muscle involved in the training. All training sessions were supervised by a skilled kinesiologist.

Data Analysis and Interpretation

Control group data and muscle function values measured in the participant with MELAS during six repetitions of isometric LE are presented as mean ± standard deviation. Due to the descriptive nature of this single case study any specific analysis was applied to the collected data.

RESULTS

Characteristics of the Participants

One patient with MELAS and eight healthy controls were successfully enrolled in the study. In **Table 1** are displayed demographic, clinical characteristics of the participants. The patient with MELAS attended 31 (86%) of the 36 scheduled sessions of RT and no adverse events occurred either during exercise or the recovery phase of RT.

Muscle Mass and Function

As illustrated in **Table 2** after 12 weeks of RT the participant with MELAS exhibited a marked increase in body mass (1.4 kg) and thigh muscle mass (0.3 kg) coupled with no detectable change in body fat. Representative images of superimposed twitches evoked in the participant with MELAS and a healthy control during the MVCs of LE are displayed in **Figure 1A**. Representative EET tracings in the LE muscle of the participant with MELAS and a healthy control are displayed in **Figure 1B**. After the training

period, the participant with MELAS exhibited an increase in MVC (5.5 N·m), EET (2.1 N·m) and VMA (13.1%). Interestingly, by normalizing MVC and EET for the thigh muscle mass all PRE to POST differences were conserved (**Table 2**).

In vivo Muscular Mitochondrial Respiration Capacity

Representative tracing of mVO_2 kinetics in a healthy control and the participant with MELAS are displayed in **Figure 2**, Panels A and B, respectively. As illustrated in **Table 2** after 12 weeks of RT the participant with MELAS exhibited no detectable changes in the end-exercise mVO_2 (0.02 mM min^{-1}), Δ mVO_2 (0.09 mM min^{-1}), while there was a marked amelioration in the τ mVO_2 (Δ70.2 s).

DISCUSSION

Although RT has been shown to be feasible and likely reduce some effects of skeletal muscle disuse in patients with mitochondrial dysfunction, the effects of RT on the neuromuscular and mitochondrial function in MELAS are not established. In the present study we investigated the effects of RT on skeletal muscle and mitochondrial function in a 21 years old male patient with MELAS. In accordance with our hypothesis the main findings of this study were: (1) After the training period the locomotor-limb function was significant increased, exhibiting strong ameliorations in MVC (5.5 N·m), EET (2.1 N·m) and (2) These ameliorations in muscle function were coupled with structural gains of muscle mass in the locomotor limbs (13%) and a significant improvement of muscle recruitment, VMA (13%). Interestingly, the *in vivo* muscular respiratory capacity (τ mVO_2) was also partially ameliorated after the RT. These promising results suggest that RT is an effective exercise approach in order to improve neurophysiological factors and muscles mass, in patients with MELAS.

TABLE 2 | Effects of resistance training (RT) on body composition, muscle function, *in vivo* and *in vitro* mitochondrial respiration capacity in a subject with MELAS.

	MELAS		CTRL
	PRE	POST	
Body composition			
Body mass (kg)	36.2	37.6	70.7 ± 5.1
Body fat (%)	30.5	30.8	11.9 ± 2.5
Thigh muscle mass (kg)	2.11	2.21	6.85 ± 0.58
Muscle function during 6 repetitions of isometric LE			
MVC (Nm)	48.8 ± 4.4	54.3 ± 1.1	173 ± 15.4
EET (Nm)	11.6 ± 1.5	13.7 ± 0.9	41 ± 6.3
nMVC (Nm·kg^{-1})	23.1 ± 2.1	24.6 ± 0.5	25.2 ± 0.8
nEET (Nm·kg^{-1})	5.5 ± 0.7	6.2 ± 0.4	6.4 ± 0.5
VMA (%)	68.2 ± 7.9	81.3 ± 4.9	94.2 ± 3.1
In vivo mitochondrial respiration capacity			
End exercise mVO_2 (mM·min^{-1})	1.99	2.01	9.82 ± 3.3
Δ mVO_2 (mM·min^{-1})	1.71	1.79	8.61 ± 2.5
τ mVO_2 (s)	94.7	24.5	21.3 ± 3.2

Data of the control participants (CTRL) and muscle function values are given as mean ± standard deviation. MELAS, Mitochondrial encephalomyopathy, lactic acidosis, and recurrent stroke-like episodes syndrome; MVC, maximal voluntary contraction; EET, electrical evoked twitch; nMVC, maximal voluntary contraction normalized by thigh muscle mass; nEET, electrical evoked twitch normalized by thigh muscle mass; VMA, voluntary activation; mVO_2, muscle oxygen consumption.

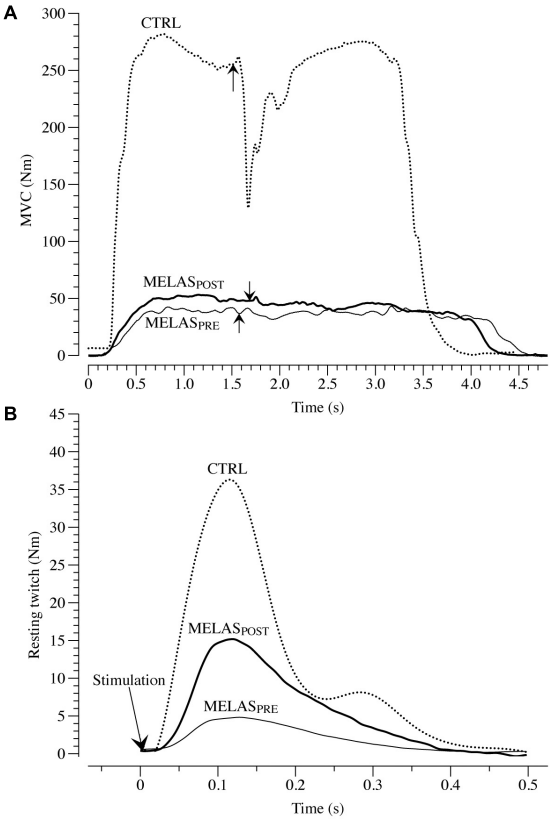

FIGURE 1 | Maximal voluntary contraction (MVC) and electrical evoked resting twitch (EET) characteristics. **(A)** Presents example tracings of the superimposed twitch technique utilized to determine muscle voluntary activation in leg extensors (LE) in a patient with mitochondrial encephalomyopathy, lactic acidosis, and recurrent stroke-like episodes syndrome (MELAS). The superimposed twitches (arrows) were imposed at the highest volitional steady-state torque. Representative examples of EET torque-time curves from LE are illustrated in **(B)**. $MELAS_{PRE}$ and $MELAS_{POST}$ represents force-tracing in a patient with MELAS before and after 12 weeks of resistance training. Dashed line represent an example tracing of MVC **(A)** and EET **(B)** of a healthy control (CTRL).

Structural, Neuromuscular and Muscle Respiratory Capacity in MELAS

As expected, before the exercise training the muscle mass of locomotor limbs was extremely reduced in the patient with MELAS as indicated by the limited thigh muscle mass

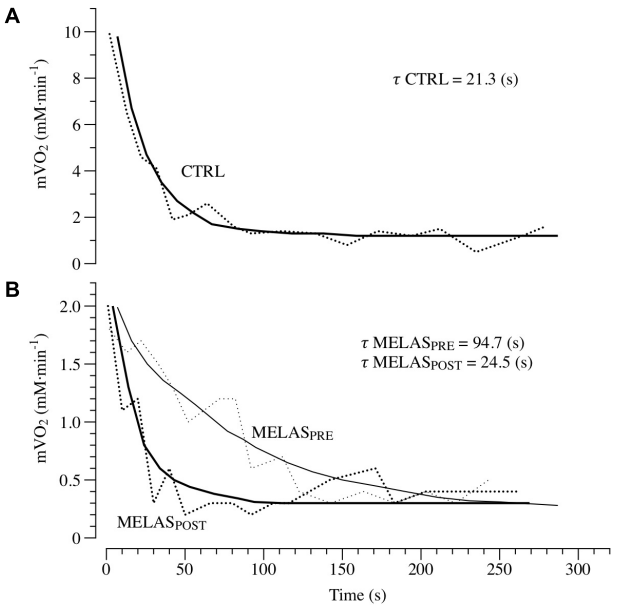

FIGURE 2 | *In vivo* mitochondrial respiration capacity. Panels **(A,B)** presents examples of the oxidative capacity measured by NIRS in the plantar flexor of a healthy 21-year-old male (CTRL) and a patient with MELAS before (MELAS$_{PRE}$) and after (MELAS$_{POST}$) 12 weeks of resistance training. The mVO$_2$ recovery data are fit to an exponential (continuous lines) to estimate the recovery *k*. The time constant (τ) is the reciprocal of the rate constant *k* (τ = 1/k).

(MELAS: 2.11 kg; CTRL: 6.85 ± 0.58). This limitation of skeletal muscle mass was coupled with a severe reduction of MVC (MELAS: 48.8 ± 4.4 Nm; CTRL: 173 ± 15.4 Nm), and EET (MELAS: 11.6 ± 1.5 Nm; CTRL: 41 ± 6.3 Nm). Moreover, maximal voluntary activation was severely reduced (MELAS: 68.2 ± 7.9%; CTRL: 94.2 ± 3.1%). Overall these results are comparable of those obtained in oldest-old and mobility limited individuals (Venturelli et al., 2015, 2018), suggesting that this reduction of muscle mass and force was partially due to the direct effect of MELAS and the partial disuse of the locomotor muscles. Moreover, muscle respiratory capacity was substantially lower in the patient with MELAS as indicated by the slower mVO$_2$ time constant (MELAS: 94 s; Controls = 21.3 ± 3.2). In agreement with this result, a twofold slower PCr recovery time constant measured by ^{31}P Magnetic Resonance Spectroscopy has previously been documented in a female patient suffering of MELAS compared to controls (Szendroedi et al., 2009). Compared with other studies examining muscle respiratory capacity *in vivo* in various patients' populations, the value reported here in this individual with MELAS are also on the lower end of the spectrum. For instance, faster Time Constant (∼30–60 s) assessed by NIRS has been documented in the lower limb muscle of patients with Cystic Fibrosis (Erickson et al., 2015), Amyotrophic Lateral Sclerosis (Ryan et al., 2014b), and Multiple Sclerosis (Harp et al., 2016). In fact, the value observed here in our patient is similar to those reported in individuals with Spinal Cord Injury [∼85 s, (Erickson et al., 2017)], which due to the denervation of the muscle exhibit an extreme level of deconditioning and severe muscle atrophy. Together, these findings suggest severe functional abnormalities of the skeletal muscle mitochondria, which would explain the high susceptibility to the development of type 2 diabetes in these patients (Velho et al., 1996; Becker et al., 2002; Stark and Roden, 2007).

Neuromuscular and Structural Effects of RT

Previously, Taivassalo et al., 1998, 1999, 2006 and Taivassalo and Haller (2004, 2005) and lately Murphy et al. (2008) reported a clear physiological rationale for the implementation of RT in the standard rehabilitation program for patients with mitochondrial dysfunctions. Specifically, these studies revealed a significant increase in leg muscle strength following RT (Murphy et al., 2008) and the results of the current study are in agreement with these previous investigations. Moreover, the present findings advance the knowledge on the interactions between neuromuscular and structural adaptations in response to RT in a rare pathology such the MELAS. The data from the current investigation indicate that RT generated positive effects on muscle structure and function. Specifically, we observed an increase in both neuromuscular activation and muscle mass of the limbs interested by the training (**Table 2** and **Figure 1**). Moreover, the nMVC, nEET, and VMA indexes, suggests that after RT the gain of force was primarily determined by ameliorations of the neuromuscular function rather than hyper-trophy of the skeletal muscle. This RT-induced amelioration in the neuromuscular function was likely associated by changes in cortical function such as decrease in inhibition and increased activity in many areas of the cerebral cortex. Moreover, it is reasonable to assume that RT played a significant role in the amelioration of voluntary force production in the participant with MELAS likely due to greater intra and intermuscular recruitment.

Effects of RT on Mitochondrial Respiration

It is well established that RT increases skeletal muscle force through the interplay of neuromuscular adaptations and the increase in cross-sectional area of the muscle. However, recent evidence suggest that mitochondrial adaptations can also occur (Murphy et al., 2008). Specifically, Murphy et al. (2008) revealed RT-related amelioration of VO$_2$peak, coupled with decreased in COX-deficient cells and increased COX activity, suggestive of improved mitochondrial function within skeletal muscle (Taivassalo et al., 2001; Taivassalo and Haller, 2004, 2005). Interestingly, short-term RT has also been reported to increase skeletal muscle respiratory capacity measured *in vitro* and *in vivo* in young and older individuals (Jubrias et al., 2001; Pesta et al., 2011). The results of the current study give further credence to the hypothesis that RT can be a potent stimulus to induce mitochondrial adaptations in healthy populations and patients

with MELAS. As illustrated in **Figure 2**, the time constant of mVO_2 was drastically shortened from ∼94 s at baseline to ∼25 s post training, i.e., a value similar to the control group (∼21 s). Given the impairment in glucose control and severe exercise intolerance associated with this disease, these findings provide a proof of concept that RT represent a safe and effective training method to induce metabolic adaptations in the skeletal muscle of patients with MELAS. The *in vivo* approach used herein to measure muscle respiratory capacity with NIRS assess the integrated function of muscle O_2 transport and utilization. Therefore, while it is unlikely that improvements in the convective or diffusive components of O_2 delivery occurred with this type of training, this possibility cannot be entirely ruled out. Based upon previous studies reporting both an increase in mitochondrial content and function (Jubrias et al., 2001; Pesta et al., 2011), and given the magnitude of the change in muscle respiratory capacity observed here, it is likely that both structural and functional changes contributed to this improvement. Further studies using *in vitro* measurements of mitochondrial content and respiratory function are therefore warranted to clarify the exact mechanism contributing to this improvement in muscle respiratory capacity *in vivo*. It is important to note, that the muscle metabolic abnormalities observed in the patient with MELAS are likely due to the disuse of locomotor limbs but also primarily affected by an intrinsic metabolic problem related to MELAS, and unfortunately partially corrigible with increased physical activity. This result is partially in contrast with previous investigation reporting the disappearance of neuromuscular (Gruet et al., 2016) or metabolic (Decorte et al., 2017) abnormalities in physically active patients with cystic fibrosis.

CONCLUSION

To conclude, this study suggests a preserved plasticity in the skeletal muscle of a patient with MELAS. More importantly, Resistance Training appears to be a safe and effective method to increase skeletal muscle function in this patient population, and this effect is mediated by both neuromuscular and mitochondrial adaptations. However, particular attention and caution is needed in the interpretation of the data of this single case study and further studies are warranted including larger sample of patients.

ETHICS STATEMENT

For this case study the participant caregiver provided written informed consent.

AUTHOR CONTRIBUTIONS

MV and FS had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. MV, FS, FV, FR, and DR designed and conducted the study. MV, FV, SS, CF, AP, NS, GL, EC, CM, and VC collected, analyzed, and interpreted the data. All authors reviewed and approved the final manuscript.

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ORIGINAL RESEARCH
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Circulating Tumor Necrosis Factor Alpha May Modulate the Short-Term Detraining Induced Muscle Mass Loss Following Prolonged Resistance Training

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Introduction: Tumor necrosis factor alpha (TNFα) is a pro-inflammatory cytokine that has been shown to modulate muscle mass, and is responsive to exercise training. The effects of resistance training (RT) followed by a short period of detraining on muscle size, architecture and function in combination with circulating TNFα levels have not been previously investigated in a young, healthy population.

Methods: Sixteen participants (8 males and 8 females) were randomly assigned to a training group (TRA; age 20 ± 3 years, mass 76 ± 7 kg), whilst fourteen participants (7 males and 7 females) age 22 ± 2 years, mass 77 ± 6 kg were assigned to a control group (CON). Measures of vastus lateralis (VL) muscle size (normalized physiological cross-sectional area allometrically scaled to body mass; npCSA), architecture (fascicle length; L_F, pennation angle Pθ), strength (knee extensor maximal voluntary contraction; KE MVC), specific force, subcutaneous fat (SF) and circulating TNFα were assessed at baseline (BL), post 8 weeks RT (PT), and at two (DT1) and four (DT2) weeks of detraining.

Results: Pooled BL TNFα was 0.87 ± 0.28 pg/mL with no differences between groups. BL TNFα tended to be correlated with npCSA ($p = 0.055$) and KEMVC ($p = 0.085$) but not specific force ($p = 0.671$) or SF ($p = 0.995$). There were significant ($p < 0.05$) increases in npCSA compared to BL and CON in TRA at PT, DT1, and DT2, despite significant ($p < 0.05$) decreases in npCSA compared to PT at DT1 and DT2. There were significant ($p < 0.05$) increases in L_F, Pθ and KE MVC at PT but only L_F and torque at DT1. There were no significant ($p > 0.05$) changes in SF, specific force or TNFα at any time points. There was a significant correlation ($p = 0.022$, $r = 0.57$) between the relative changes in TNFα and npCSA at DT2 compared to PT.

Discussion: Neither RT nor a period of short term detraining altered the quality of muscle (i.e., specific force) despite changes in morphology and function. TNFα does not appear to have any impact on RT-induced gains in muscle size or function, however, TNFα may play a role in inflammatory-status mediated muscle mass loss during subsequent detraining in healthy adults.

Keywords: cytokine, inflammation, muscle architecture, specific force, young

INTRODUCTION

Reduced levels of skeletal muscle mass and strength are associated with many chronic diseases and increased mortality in humans (Rantanen, 2003; Oterdoom et al., 2009; Kalyani et al., 2014; Bowen et al., 2015), understanding the fundamental adaptation of skeletal muscle is therefore, key to optimizing health and longevity. Skeletal muscle displays remarkable plasticity with its ability to alter its phenotype in response to mechanical/metabolic stimuli or lack thereof, with an increase (hypertrophy) or decrease (atrophy) in muscle fiber size, respectively (Coffey and Hawley, 2007; Wackerhage et al., 2018). Resistance exercise is well established as a potent stimulus for acutely altering muscle protein synthetic rates (MacDougall et al., 1995), and following repeated resistance training (RT) sessions over a period of time, this stimulus ultimately results in an increase in skeletal muscle mass and strength [e.g., (Moritani and DeVries, 1979; Young et al., 1983; Frontera et al., 1988; McMahon et al., 2012, 2014, 2018)]. During a period of detraining (DT) however, preceding gains in muscle fiber size are rapidly diminished. For example, in a mouse model, Jespersen et al. (2011), demonstrated that following 90 days of RT, almost 50% of the RT-induced increase in muscle fiber size was lost after only 10 days of DT. In humans, detraining in the form of 5–6 weeks immobilization, following 5–6 months of RT, resulted in a 41% loss of RT-induced gains in elbow flexor strength, and 33 and 25% reduction in fast/slow twitch muscle fiber size, respectively, in healthy male subjects (MacDougall et al., 1980). Early work from Häkkinen and Komi (1983) demonstrated that following 16 weeks of strength training in young, healthy males, muscle strength and integrated electromyography (iEMG) of the leg extensors were significantly increased. Following a subsequent 8 week detraining period, the significant reductions in muscle force were accounted for by a reversal of the preceding RT induced gains in neuromuscular variables. More recently, Blazevich et al. (2007) reported that following 10 weeks of either concentric or eccentric RT in young men and women, there were significant improvements in muscle volume, physiological cross-sectional area (pCSA), architecture and training mode specific strength. Following 3 months of detraining, there was a significant, contraction mode specific, decrement in strength (eccentric only) and interestingly non-significant changes in hypertrophic and architectural measures. Despite this evidence in RT and subsequent DT studies of longer durations, which may reflect different patterns of adaptation to RT and DT, periods of DT following RT and the associated alterations to muscle mass and function to date have been poorly characterized in young, healthy adults following short-term detraining such as 1–4 weeks' post-RT. To the authors' knowledge, there are only a few studies that have been published previously that have reported changes to muscle mass and strength following short-term DT (≤4 weeks) following a RT only program (i.e., not part of a concurrent RT and endurance training program) (Kubo et al., 2010; McMahon et al., 2012; Yasuda et al., 2015). However, large methodological discrepancies make these studies incomparable due to factors such as muscle groups analyzed, length of the preceding training period, measurement points during detraining, and differences in muscle size and strength measurements. Our paper has previously demonstrated that muscle size (anatomical cross-sectional area [aCSA]) and maximal voluntary contraction (MVC) torque is significantly elevated above baseline following 8 weeks of RT and remained elevated following a further 2 and 4 weeks of detraining (McMahon et al., 2012), although these varied slightly depending on the training group. Two factors that are critical in fully describing muscles' characteristics are physiological cross-sectional area (the area of muscle at right angles to the longitudinal axis of the fibers) and the muscle's specific force (normalization of force per unit pCSA) which, in combination with neural factors (Moritani and DeVries, 1979; Del Vecchio et al., 2019) provide a true representation of the muscle size and intrinsic force generating capacity. Neither pCSA nor specific force have been investigated in short-term (≤4 weeks) detraining studies, with specific force, to the author's knowledge, not within any length of detraining studies following a period of RT.

In addition to RT acting as stimuli for phenotype changes, the endocrine system also plays a critical role in phenotype expression by having the ability to tip the balance of muscle cellular anabolism and catabolism through influence of various growth factors and cytokines (Solomon and Bouloux, 2006). One such cytokine (or myokine when secreted from muscle) that plays an integral role in altering muscle state is tumor necrosis factor alpha (TNFα). TNFα is a pro-inflammatory cytokine that is associated with the modulation of muscle tissue loss, particularly in pathological disease such as cancer cachexia (Balkwill, 2006) and Sarcopenia (Visser et al., 2002). For example, high levels of circulating IL-6 and TNFα have been associated with lower muscle mass and strength in well-functioning older adults (Visser et al., 2002). Loss of specific muscle proteins such as myosin heavy chain (MHC) content are modulated by TNFα- mediated effects on the p38 MAPK and nuclear factor-κB (NF-κB) pathways of the ubiquitin-proteasome pathway (Li et al., 1998, 2005; Li and Reid, 2000). Li et al. (1998) demonstrated that following 72 h of 1–6 ng/mL of TNFα treatment on differentiated mouse skeletal muscle C2C12 myotubes, there were significant protein content losses. Furthermore, concentrations as low as 1–3 ng/mL induced losses of adult MHC protein in a concentration dependent

manner in primary cultures from rat skeletal muscle. These losses were not associated with a decrease in muscle DNA content or a decrease in MHC protein synthesis, which is reflective of chronic muscle wasting (Li et al., 1998). In a subsequent study from the same group (Li et al., 2005) also demonstrated that following intraperitoneal injection of 6 ng/mL of TNFα, Atrogin-1/MAFBx expression increased by 76.2 ± 17.1% after 4 h and 87.7 ± 10.1% after 6 h in adult gastrocnemius muscles of mice. Previous research into the impact of RT on TNFα levels has been mainly conducted in only elderly or diseased populations, with only sparse data in a young population currently available. Other methodological issues within the literature is that many studies have investigated concurrent training (i.e., combinations of RT and endurance training), not singularly RT and provide little or no insight into changes in muscle mass or function. Louis et al. (2007) reported that following an acute bout of RT in young adults, TNFα mRNA was significantly increased immediately and for up to 24-h post-RT. In a study by Townsend et al. (2013) also investigating the acute response to RT, the authors showed that in resistance-trained men, circulating TNFα was significantly increased immediately post-RT compared to pre-RT. However, TNFα levels had returned to baseline within 30 min post-RT, remaining as such for a further 24 and 48 h. This is in somewhat of agreement with the study of Alves et al. (2013) who demonstrated that following either eccentric or concentric RT only, there were no acute increases in TNFα post-RT (24–96 h). Furthermore, this study also showed that following 10 RT sessions over 3 weeks, there were no significant changes in circulating TNFα 24–96 h post the final RT session. Paoli et al. (2015) report that following 8 weeks of upper body RT in combination with either a high-or-low protein diet, despite no significant alterations in the acute circulating TNFα response, the authors noted chronic RT could elevate both the basal and RT-induced response of circulating TNFα. The authors of this previous study, however, report neither changes in muscle mass or strength. Furthermore, data from Bruunsgaard et al. (2004) demonstrate that muscle strength after 12 weeks of RT in 86–95 year olds is inversely correlated with TNFα receptor content, showing that the TNFα system can also impact on muscle function. Currently there are no investigations on TNFα levels and concomitant changes in muscle size and strength in younger adults following a sustained RT program only, and following a period of short-term detraining.

Therefore, the aims of this study were to (1) investigate the effects of RT and DT on muscle size and function, (2) describe changes in TNFα levels following RT and DT, and (3) probe whether there is any link between changes in muscle size and function and circulating TNFα levels in a young, healthy population.

MATERIALS AND METHODS

Participants

Thirty young participants recruited from the local university campus, gave written informed consent to participate in the study in accordance with the Declaration of Helsinki. All procedures and experimental protocols were approved by the Manchester Metropolitan University Cheshire Campus ethics committee. Exclusion criteria included the presence of any known musculoskeletal, neurological, inflammatory, or metabolic disorders or injury. Participants took part in recreational activities such as team sports and had either never taken part in lower limb RT or had not done so within the previous 12 months. Each participant completed a physical activity diary, outlining that they each habitually completed 3–5 h of non-resistance based moderate physical activity per week. Sixteen participants were randomly assigned to a training group (TRA) age 20 ± 3 years, mass 76 ± 7 kg), whilst fourteen participants (7 males and 7 females) age 22 ± 2 years, mass 77 ± 6 kg were assigned to a control group (CON).

Study Design

The study design was convenience sampling, with participants separated into groups by random allocation to one of two groups (i.e., training or control). Following familiarization with laboratory procedures at least 1 week prior to testing proper, participants were assessed for muscle morphology, architectural and functional properties and TNFα at baseline (week 0). Measurements were repeated after 8 weeks of RT (post-training [PT]), week 10 following 2 weeks of detraining (DT1) and week 12 following a further 2 weeks detraining (DT2).

Resistance Training

Resistance training was performed three times per week for 8 weeks at 80% of 1 repetition maximum (1RM) on the knee extensor complex. Exercises included the back squat, leg press, leg extension (Technogym, Berkshire, United Kingdom), lunge, Bulgarian split squat and Sampson chair. All exercise sessions were supervised by a member of the research team. Participants completed two familiarization sessions at 70%1RM prior to commencing the RT program. 1RMs were measured at baseline and every 2 weeks, with loading weight progressed. Each training session consisted of four exercises with participants performing three sets of 10 repetitions per exercise for the first 4 weeks, and four sets of eight repetitions per exercise thereafter. Training records were diligently completed during sessions. Following RT, participants returned to habitual daily activities with no form of exercise training permitted for the detraining period (4 weeks).

Assessment of Muscle Morphology

All muscle morphological and architectural measures were taken at rest with each participant seated in an upright position on an isokinetic dynamometer (Cybex, CSMi, MA, United States). After calibration, each participant was positioned with a hip angle of 80° (straight back 90°) and knee at 90° knee flexion (straight leg 0°). All measurements were determined using ultrasonography (AU5, Esaote Biomedica, Genoa, Italy) at rest with a 40 mm probe, with images captured at 25 Hz using a digital video recorder (Tevion, Medion Australia Pty Ltd.,

St Leonards, Australia). The measurement sites were 25, 50, and 75% of femur length. Femur length was defined as the line passing from the greater trochanter to the central palpable point of the space between the femur and tibia heads when the knee was flexed at 90°. Vastus lateralis fascicle pennation angle (θ) was measured as the angle of fascicle insertion into the deep aponeurosis. Images were obtained perpendicular to the dermal surface of the VL and oriented along the midsagittal plane of the muscle. The transducer was then aligned in the fascicle plane to capture an optimal number of clearly demarked fascicles. Images were taken at 25, 50, and 75% of the total femur length (as described below) and 50% of muscle width at each point (where 50% muscle width is defined as the midpoint between the fascia separating the VL and rectus femoris, and fascia separating the VL and biceps femoris muscles). Fascicle length was defined as the length of the fascicular path between the deep aponeurosis and superficial aponeurosis of the VL. The majority of fascicles extended off the acquired image, where the missing portion was estimated by linear extrapolation. This was achieved by measuring the linear distance from the identifiable end of a fascicle to the intersection of a line drawn from the fascicle and a line drawn from the superficial aponeurosis. This method has been shown to produce reliable results previously (Blazevich et al., 2007). All images were analyzed and measured using Image J v.1.43c (National Institutes of Health, Bethesda, MD, United States). Subcutaneous fat was estimated using the same images as taken for muscle architecture and analyzed at 50% femur length only. After calibration in Image J to coincide with the scale of the ultrasound image, a line from the top to the bottom of the layer of fat visualized was drawn at three regular intervals on the ultrasound image. The average lengths of these three lines were taken to estimate the average thickness of the subcutaneous fat layer in millimeters. Care was taken not to deform or compress the subcutaneous fat with minimal pressure applied to the dermal surface with the ultrasound probe.

For a full description of aCSA methods please see Reeves et al. (2004a) The aCSA was measured with the probe aligned in the axial plane. Echo-absorptive tape was placed at regular intervals (∼3 cm) along the muscle width at each site so that when the probe was placed on the leg, two distinct shadows were cast on the ultrasound image. Therefore, each ultrasound image provided a section of VL within the boundaries set by the two shadows and fascia surrounding the muscle. Each of these sections was analyzed for the total area using Image J to provide a total aCSA at that particular site.

The measurement techniques used for the calculation of physiological cross-sectional area of the vastus lateralis (VL) muscle in the current study has been previously documented elsewhere (Reeves et al., 2004a; McMahon et al., 2014). Using the aCSA measures, muscle volume was then calculated using the truncated cone method, which has been validated in a number of previous studies (Esformes et al., 2002; Reeves et al., 2004b). VL pCSA was calculated by dividing muscle volume by fascicle length (Reeves et al., 2004b). Allometric scaling was then used to normalize (npCSA) to body mass (Jaric et al., 2005).

Knee Extensor Maximal Voluntary Contraction (KE MVC) and Vastus Lateralis Specific Force

Prior to this assessment, a number of measures were taken to minimize inaccuracies during the dynamometer assessments, thereby counteracting any potential effect of soft tissue compliance, dynamometer alignment, as well as gravitational forces. With these precautions in place, maximal isometric knee extension torque was measured with the knee at 70° knee flexion (full knee extension = 0°) on the right leg of all participants, corresponding to the angle of peak torque. After a series of warm up trials consisting of ten isokinetic contractions at $60°.s^{-1}$ at self-perceived 50–85% maximal effort, participants were instructed to rapidly exert maximal knee extensor isometric force (maximal voluntary contraction, KE MVC) against the dynamometer lever arm. Joint torque data traces were displayed on the screen of a MacBook Air computer (Apple Computer, Cupertino, CA, United States), which was interfaced with an A/D system (Acknowledge, Biopac Systems, Santa Barbara, CA, United States) with a sampling frequency of 2000 Hz. Isometric contractions were held for ∼2–3 s at the plateau with a 60 s rest period between contractions. Peak torque was expressed as the average of data points over a 500 ms period at the plateau phase (i.e., 250 ms either side of the instantaneous peak torque). The peak torque of three extensions was used as the measure of torque in each participant.

A pair of Ag-AgCl electrodes (Neuroline 720, Ambu, Denmark), were placed on clean, shaved, and abraded skin, at 50% of femur length, in the mid-sagittal plane of the biceps femoris. The reference electrode (Blue sensor L, Ambu, Denmark) was placed on the lateral tibial condyle. The raw EMG signal was preamplified (MP100, Biopac Systems Inc., United States), amplified × 1000 (MP100, Biopac Systems Inc., United States), bandpass filtered between 10 and 500 Hz (Biopac Systems, United States) with a notch at 50 Hz, and sampled at 2000 Hz. All EMG and torque signals were displayed in real time in AcqKnowledge software (Biopac systems Inc., United States) via a PC. Two maximal knee flexion contractions were carried out to obtain the EMG at maximal flexion torque. The root mean square (RMS) EMG activity was averaged for a 500 ms period (average of 1.5 ms moving windows) which coincided with the plateau of peak KE MVC torque. To reiterate, the EMG of the long head of the biceps femoris muscle was measured to ascertain the level of antagonist muscle co-contraction during knee extensor MVC. The biceps femoris torque during a knee-flexion contraction was calculated as described by McMahon et al. (McMahon et al., 2014) whereby a linear relationship between BF EMG and KE MVC torque is assumed, thus enabling the quantification of the "pull back torque" during knee extensions, and ultimately, the total forces experienced by the patella tendon (Pearson and Onambele, 2005, 2006).

Muscle specific force was calculated in multiple steps as previously reported (Reeves et al., 2004b). Briefly, VL fascicle

force was calculated by dividing the estimated VL muscle force by the Cosθ angle of pennation at 50% femur length. Specific force of the VL muscle was calculated at the knee joint angle where maximal fascicle force peaked, which corresponded to the knee joint angle of 70° before and after training. Specific force was calculated by dividing fascicle force by pCSA.

Circulating Tumor Necrosis Factor Alpha (TNFα) Levels

Pre-, post-training and during detraining, following an overnight fast, (∼10 h), participants reported to the laboratory between 9 and 11am. 5 mL blood samples were collected from the antecubital vein of the forearm, placed in a crushed ice bed for 1.5 to 2-h, and then centrifuged at 4°C for 10 min at 4,800 rpm, with the supernatant being removed and stored in at least two aliquots in eppendorf at −20° Celsius for later analysis. TNFα was analyzed using the standard enzyme-linked immuno-sorbent assay (ELISA) procedure, as described previously (McMahon et al., 2013). Post-training samples were taken 3–4 days post final training session, at the same time-of-day as the pre-training sampling for each participant, with detraining samples being taken twice fortnightly after the post-training sample date. The laboratory tests were timed to avoid diurnal variability or acute exercise-induced cytokine fluctuations. In CON group, TNFα analysis, $n = 6$ (3 males, 3 females) with TRG $n = 16$.

Statistical Analyses

Data were parametric (as determined through Shapiro Wilk and Levene's tests) and were therefore analyzed using a mixed-design repeated measures ANOVA. The within factor was the phase of training (i.e., weeks 0, 8, 10, and 12) and the between factor was group (TRG or CON). All data are presented as mean ± SD. Statistical significance was set with alpha at $p \leq 0.05$. In terms of the sample size in this study, the average statistical power of the measured muscle parameters (VLCSA, pennation angle, fascicle length, and KE MVC was statistically adequate at beta = 0.89.

RESULTS

There were no significant differences ($p > 0.05$) between TRA and CON groups (independent t-tests) in any of the measured muscle variables, at baseline. Pooled baseline TNFα was 0.87 ± 0.28 pg/mL with no differences between groups. There was a tendency for baseline TNFα to be correlated with npCSA ($p = 0.055$) and also KEMVC ($p = 0.085$), however, there was no correlation with specific force ($p = 0.671$) or SF ($p = 0.995$).

Muscle Morphology

There were significant absolute increases ($p < 0.01$) in npCSA compared to baseline at all time points (PT, DT1, and DT2) in TRA (**Figure 1**), with no changes in CON ($p > 0.05$). There were also significant relative changes in npCSA at all time points (26 ± 23%, 18 ± 20%, and 12 ± 19% for PT, DT1, and DT2, respectively) compared to baseline and controls ($p < 0.05$). When compared to PT, there were significant absolute (**Figure 1**) and relative (−6 ± 8% and −10 ± 8%) reductions in npCSA in TRA at DT1 and DT2, respectively, and versus controls ($p < 0.05$).

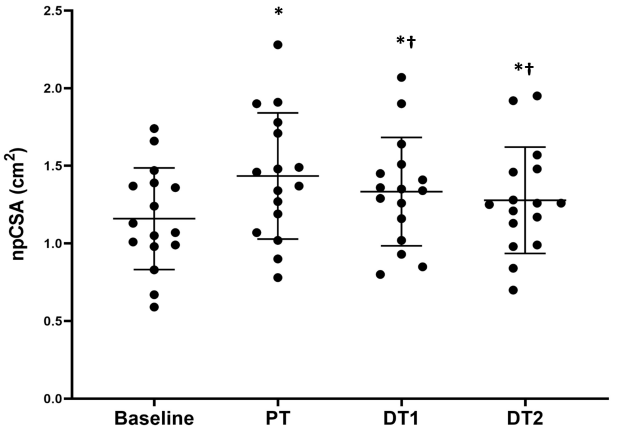

FIGURE 1 | Changes in vastus lateralis normalized physiological cross-sectional area (npCSA) in TRA group at baseline, post-training (PT), detraining 1 (DT1), and detraining 2 (DT2). *Significantly different to baseline $p < 0.001$, †Significantly different to PT $p < 0.05$.

All Pθ data are presented in **Table 1**. There was a significant effect of time on absolute changes in Pθ, with the only significant difference found between Pθ at PT and DT2 in within TRA changes, with no significant difference between TRA and CON at any time point. There was a significant ($p < 0.05$) change in Pθ at PT compared to baseline and CON but not at DT1 or DT2 in TRA, with no changes ($p > 0.05$) in CON at any time point. There were no significant ($p > 0.05$) differences in Pθ at DT1 or DT2 compared to PT in TRA.

All L_F data are presented in **Table 1**. There were significant ($p < 0.01$) absolute increases in L_F at PT and DT1 compared to baseline and CON, but not at DT2 ($p > 0.05$) in TRA, with no changes in CON at any time point. There were also significant ($p < 0.05$) absolute decreases in L_F at DT1 and DT2 compared to PT. There were significant ($p < 0.01$) relative increases in L_F at PT and DT1 compared to baseline and CON, but not at DT2 ($p > 0.05$) in TRA, with no relative changes in CON at any time point. There were also significant ($p < 0.05$) relative decreases in L_F at DT1 and DT2 compared to PT.

There were no significant absolute or relative changes in SF compared to baseline at any time points (PT, DT1, and DT2) in TRA or CON ($p > 0.05$, **Table 1**). There were no correlations between subcutaneous fat levels and TNFα levels at any point.

Knee Extensor Maximal Voluntary Contraction (KE MVC) and Vastus Lateralis Specific Force

All torque data are presented in **Table 1**. There were significant absolute and relative increases ($p < 0.05$) in KE MVC at PT and DT1 compared to baseline in TRA with no changes in CON. There were no significant absolute decreases in KE MVC at DT1 ($p > 0.05$) although there were significant absolute decreases in KE MVC at DT2 ($p < 0.01$) compared to PT in TRA with no

TABLE 1 | Functional and architectural properties.

Variable	Absolute				Relative				
	BL	PT	DT1	DT2	PT vs. BL %	DT1 vs. BL %	DT2 vs. BL %	DT1 vs. PT %	DT2 vs. PT %
Specific force (N/cm^2)									
TRA	20.9 ± 5.4	20.7 ± 4.4	20.7 ± 4.5	21.0 ± 4.7	2 ± 20	2 ± 19	3 ± 22	−1 ± 9	−1 ± 6
CON	22.3 ± 7.4	22.8 ± 7.6	23.0 ± 7.1	22.8 ± 7.8	2 ± 6	1 ± 5	2 ± 5	0 ± 3	2 ± 6
KE MVC (N·m)									
TRA	248 ± 88	275 ± 94*	266 ± 92*	259 ± 91†	13 ± 18*	9 ± 19*	6 ± 17	−4 ± 5	−6 ± 5†
CON	267 ± 76	259 ± 82	262 ± 71	268 ± 84	−1 ± 11	−1 ± 9	0 ± 14	2 ± 8	3 ± 13
VL fascicle force (N)									
TRA	1068 ± 365	1266 ± 347	1163 ± 339	1159 ± 313	–	–	–	–	–
CON	1723 ± 402	1698 ± 388	1768 ± 342	1788 ± 422					
VL pennation angle (°)									
TRA	16.0 ± 1.4	16.5 ± 1.3	16.2 ± 1.1	15.6 ± 1.4	4 ± 6*	2 ± 8	−2 ± 8	−2 ± 4	−5 ± 4
CON	14.1 ± 6.3	14.0 ± 6.2	13.9 ± 6.2	14.0 ± 6.1	0 ± 4	−1 ± 5	0 ± 4	−1 ± 2	0 ± 1
VL fascicle length (mm)									
TRA	101 ± 16	116 ± 16*	112 ± 14*†	106 ± 13†	16 ± 9*	11 ± 9*	6 ± 8	−4 ± 3†	−9 ± 5†
CON	108 ± 16	111 ± 15	112 ± 16	111 ± 17	3 ± 6	6 ± 6	3 ± 7	2 ± 3	−1 ± 3
Sub fat (mm)									
TRA	7.7 ± 6.2	6.9 ± 5.3	7.3 ± 5.8	7.7 ± 6.0	−9 ± 14	−1 ± 18	6 ± 26	10 ± 26	19 ± 40
CON	6.1 ± 4.7	6.4 ± 5.3	6.5 ± 5.3	6.4 ± 5.3	3 ± 12	8 ± 18	3 ± 12	5 ± 18	0 ± 1

*BL, baseline; PT, post-training; DT1, detraining 1; DT2, detraining 2; TRA, training group; CON, control group; KE MVC, knee extensor maximal voluntary contraction; Sub fat, subcutaneous fat; VL, vastus lateralis. *Significantly different compared to baseline, †Significantly different to post-training.*

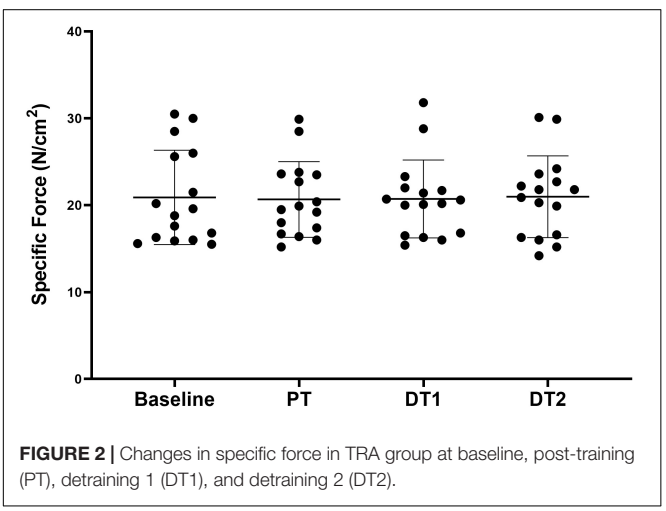

FIGURE 2 | Changes in specific force in TRA group at baseline, post-training (PT), detraining 1 (DT1), and detraining 2 (DT2).

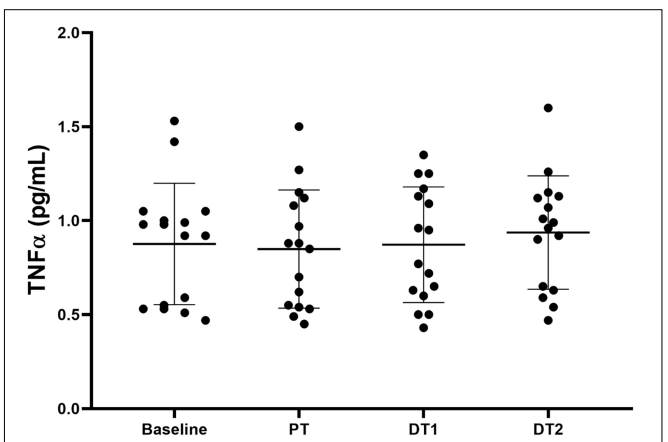

FIGURE 3 | Changes in TNFα in TRA group at baseline, post-training (PT), detraining 1 (DT1), and detraining 2 (DT2).

changes in CON. There was also a significant relative decrease in KE MVC at DT2 ($p < 0.05$) but not DT1 ($p > 0.05$) compared to PT in TRA with no changes in CON. All specific force data are presented in **Table 1**. There were no significant ($p < 0.05$) absolute or relative changes in specific force at any time point in TRA (**Figure 2**) or in CON.

Tumor Necrosis Factor Alpha (TNFα)

There were no significant ($p > 0.05$) absolute or relative changes in TNFα compared to baseline or CON in TRA at any time point (**Figure 3**). There were also no significant absolute or relative changes in TNFα compared to PT at DT1 or DT2 ($p > 0.05$). However, there was a significant correlation ($p = 0.022$, $r = 0.57$) between the relative changes in TNFα and npCSA at DT2 compared to PT.

DISCUSSION

The main findings from the current study are that (1) following 8 weeks of RT there was no effect on systemic TNFα levels, and as such the training benefits were independent of this endocrine parameter, (2) changes in TNFα levels were significantly correlated with losses in muscle mass following 4 weeks of detraining, and (3) we report for

the first time that a short period of DT does not appear to alter the quality (i.e., specific force) of the remaining skeletal muscle.

Resistance Training and TNFα Levels

The role of physical activity is apparent, when taking into account its role in systemic inflammation. Indeed physical activity is believed to play a central role in the delicate balance between so-called myokines, which are cytokines produced by contracting skeletal muscles during physical activity. Examples of these myokines are interleukin (IL)-6 and TNF-α (Pratesi et al., 2013). Interestingly, IL-6 has always been considered a pro-inflammatory cytokine, but it might display anti-inflammatory effects during exercise (Pratesi et al., 2013). TNF-α on the other hand, can both stimulate muscle growth (Chen et al., 2007) but also lipid metabolism through minimizing the accumulation of lipids in adipocytes (Wang et al., 2013). Hence, reverting to a less-active lifestyle following training would cause an imbalance, resulting in a pro-inflammatory status and the beginning of a vicious cycle including enhancement of muscle loss, accumulation of intramuscular fat and a decrease in muscle strength. This is more or less confirmed by previous studies showing that low physical activity and/or sedentary behavior is indeed associated with skeletal muscle atrophy (Degens and Alway, 2006; Bostock et al., 2013). On the contrary, physical activity can provide a physiological stimulus, making the skeletal muscle behave as an endocrine organ, counteracting inflamed aging (Pratesi et al., 2013). It is also interesting in the current study that pooled baseline TNFα levels tended to be correlated ($p = 0.055$, $r = 0.41$) with baseline npCSA in the young population. This would also reflect the evidence showing that TNFα has a dose-dependent relationship on MHC loss in skeletal muscle (Li et al., 1998). It is well established that a chronic low-grade inflammatory profile (CLIP) is associated with TNFα levels and age-related Sarcopenia in older populations (Beyer et al., 2012). Our current data suggests that there may be a possible role of pro-inflammatory cytokine modulation of muscle mass even in the younger, healthy adult. However, this is purely speculative at this point.

Previous research using young participants found that the mRNA of TNFα was significantly increased immediately and remained elevated up to 24 h post-resistance exercise (Louis et al., 2007), which is in contrast to the findings of Franchi et al. (2014) who did not detect changes in TNFα phosphorylation after concentric or eccentric resistance exercise. However, circulating TNFα levels appear to display a shorter temporal pattern. Townsend et al. (2013) showed with the acute response to RT, circulating TNFα was significantly increased immediately post-RT compared to pre-RT in resistance trained men. However, TNFα levels had returned to baseline within 30 min post-RT, remaining as such for a further 24 and 48 h. Similarly, Alves et al. (2013) demonstrated that following either eccentric or concentric RT only, there were no acute increases in TNFα post-RT (24–96 h). In addition, Peake et al. (2006) reported following submaximal and maximal lengthening contractions, no significant changes in circulating TNFα in either acute windows of 1–3 h or 1–4 days. The results from the current study show that systemic levels of TNFα are not significantly altered by an 8-week RT program. This is also in somewhat of agreement with the study of Alves et al. (2013) whom reported that following 10 RT sessions over 3 weeks, there were no significant changes in circulating TNFα 24–96 h post the final RT session. The current study is slightly in contrast with that of Paoli et al. (2015). These authors report that following 8 weeks of upper body RT in combination with either a high- or-low protein diet, chronic RT could elevate both the basal and RT-induced response of circulating TNFα. The pre-, post-RT baseline changes in circulating TNFα levels (5.9 ± 0.46 ng/L to 15.79 ± 7.4 ng/L in normal protein diet, and 6.44 ± 0.65 ng/L to 11.04 ± 2.16 ng/L in high protein diet) were not directly compared statistically, rather the authors compared differences in the acute response to RT at baseline and post 8 weeks RT. Therefore it is still unclear to the exact effects of RT on chronic circulating TNFα levels. In addition the authors of the aforementioned study, however, did not report either changes in muscle mass or strength, which further compounds the lack of clarity of what effects marginal changes in TNFα levels may have, if any at all. Using a slightly different approach, Rall et al. (1996b) used peripheral blood mononuclear cells (PBMC) subpopulations under various conditions to investigate the inflammatory response of a young adult population versus rheumatoid arthritis and healthy elderly populations to 12 weeks of progressive RT. There were no changes in TNFα production in PBMC under any condition in the young healthy adults following RT. It is worth noting that despite this, the changes in young adults' strength was significantly increased post-RT with data shown in a previous study (Rall et al., 1996a). This is in agreement with the observations of the current study with participants undergoing significant improvements in strength with no changes in TNFα. In a study by Ihalainen et al. (2018), the authors found that systemic TNFα were significantly reduced following 24 weeks of combined aerobic and RT in young men. However, due to 3-fold greater duration of training, lack of particular details around the RT program variables and the use of males only, make any comparisons between the current study and that of Ihalainen et al. (2018) very difficult. Furthermore, the acute response of TNFα to RT exercise and endurance exercise have been found to be disparate (Louis et al., 2007). These observations therefore again highlight the need for concurrent aerobic and RT programs to be treated independently from RT programs only, and that a combination of training modes (RT + aerobic training) may be more beneficial for modulating pro-inflammatory cytokines. The lack of change in TNFα levels following a RT only program are consistent with those found in previous research in a different age-based population. In that particular study, 11 middle-aged sedentary men (49 ± 5 years) completed 16 weeks of RT with no change in TNFα levels (Libardi et al., 2012). However, it is worth noting the baseline and post-RT TNFα levels were 3-fold greater than the current study and could be verging on clinical levels (Milani et al., 1996). In older populations TNFα levels have been shown to be sensitive to a RT program with

reductions in TNFα levels reported following the completion of such programs (Greiwe et al., 2001; Tomeleri et al., 2016) with baseline TNFα receptor levels inversely correlated with changes in strength (Bruunsgaard et al., 2004). Reductions in systemic TNFα following RT in older populations it must be pointed out are not universally found, with some studies also showing no alterations to TNFα levels (Onambélé-Pearson et al., 2010a,b). Therefore in contrast to the above data regarding sensitivity of TNFα to RT in older populations, it appears that following a RT program in young, healthy participants, systemic TNFα is not sensitive to RT-induced changes in muscle size and/ or function.

Interestingly, our data demonstrates that the relative decrease in muscle npCSA from post-RT to 4 weeks of detraining was significantly correlated with the change in TNFα levels over the same period, which tended to increase. Acute systemic administration of TNFα has been shown to increase the expression of ubiquitin mRNAs in soleus muscles (Llovera et al., 1997) and reduce protein synthesis rates of myofibrillar and sarcoplasmic proteins in the gastrocnemius (Lang et al., 2002). However, *in vitro* work has demonstrated that superimposition of anabolic stimuli can ablate the effects of TNFα on muscle protein loss (Guttridge et al., 2000). Therefore during a period of detraining, where there is a lack of anabolic stimuli, changes in TNFα levels may mediate the loss of muscle mass, however, further work is needed to support this. Previous work on TNFα suggests these effects are modulated through TNFα's upstream effects on the p38 MAPK and nuclear factor-κB (NF- κB) pathways of the ubiquitin-proteasome pathway (Li et al., 1998, 2005; Li and Reid, 2000).

Specific Force

Vastus lateralis muscle specific force did not change in the current study following RT. This is in contrast to the only other published study that has investigated *in vivo* muscle specific force changes following RT in young individuals. Erskine et al. (2010) demonstrated that following 9 weeks of RT, VL muscle specific force increased by 20% in 17 untrained males. The methods and RT used by Erskine et al. (2010) were very similar to those of the current study, however, importantly in the current study we did not assess muscle activation levels using the interpolated twitch technique. Despite this there is currently no consensus as to whether maximal muscle activation, whether supramaximally stimulated or not, is actually increased following a RT program (Folland and Williams, 2007). This therefore means that the current data can still be accurately representative of muscle specific force changes following RT. Additionally, the study of Erskine et al. (2010) used males only, however, in the current study 50% of the training group were females. No data is currently available on whether there are sex differences in muscle specific force, although we have previously demonstrated that males and females have similar relative muscle adaptations to a RT program, and the authors do not think this explains the differences between these studies. A reason why there were no observed changes in muscle specific force in the current study is that both the npCSA (muscle size) and torque (muscle strength) were both significantly enhanced following training, but none more so than the other. In other studies, muscle strength has increased to a larger degree than muscle size [e.g., Erskine et al. (2010)] however, due to the heterogeneous responses of individuals to changes in muscle size and strength, this relationship was not apparent in the current study. In conjunction with this, it should also be pointed out there were no correlations between changes in muscle size and strength following RT and during DT in the present study.

Changes in npCSA have not been reported previously following a short-term period of detraining such as that used in the current study, nor has specific force been reported during any detraining literature. Many human muscles, including the quadriceps are pennate muscles, and therefore aCSA measurements underestimate pCSA (Wickiewicz et al., 1983). The pCSA represents the area of sarcomeres arranged in parallel and thus the maximum amount of cross-bridges that can be formed during a contraction and is the primary determinant of maximal muscle force. Importantly, we also normalized the measured pCSA to body mass through allometric scaling which is an influential factor when comparing individuals with heterogeneous body composition. There were significant reductions in npCSA following both two ($-6 \pm 8\%$) and 4 weeks ($-10 \pm 8\%$) of DT compared to PT (mean -2.5% per week). This short term reduction in muscle mass is consistent with previous observations that used anatomical CSA measurements (McMahon et al., 2012; Yasuda et al., 2015). However, as both of these previous studies investigated the effects of two different training regimens on muscle size, effects of training and detraining were not the same between these groups. Despite the aforementioned loss of muscle mass, npCSA of the VL muscle was still significantly increased relative to baseline after 4 weeks of detraining. Again this is in agreement with the data of Yasuda et al. (2015) that showed following 6 weeks of RT and a subsequent 3 weeks of DT, pectoral and triceps brachii CSA was still improved compared to baseline. Trends for changes in strength mirrored those of npCSA in the main, with significant reductions in strength at DT1 and DT2 compared to PT. KE MVC remained greater compared to baseline at DT1, however, this had diminished by DT2. Previous data has shown that muscle strength was maintained following 1 month and up to 3 months of detraining following 3 months of RT (Kubo et al., 2010). The previous authors suggested this was in part due to neural adaptations. This may explain in part the differences observed between the studies. In the current study, our participants completed 8 weeks of RT using dynamic muscle contractions and were assed for maximal voluntary contraction (MVC) via isometric contractions. Kubo et al. (2010) participants were trained and assessed using isometric contractions with a 50% longer training duration. Therefore the neural specificity of adaptations to training and testing protocol, and longer training time may explain some of the differences (Folland and Williams, 2007). This is the first study to report muscle specific force during a period of DT of

any length. Muscle specific force accounts for many of the other extrinsic factors present in analyzing the relationship between muscle size and strength such as angle of fiber insertion to the aponeurosis, antagonist co-contraction, moment arm length etc., and requires specific measurements such as force at optimum fiber length, pCSA, muscle architecture and muscle volume for example. Specific force has been found to range between 10 and 40 $N.cm^{-2}$ in human single muscle fibers *in situ* [e.g., Degens et al. (1999)] and between 10 and 100 $N.cm^{-2}$ in humans *in vivo* (Narici et al., 1988; Maganaris et al., 2001). The muscle specific force ranged from 14.2 to 30.5 $N.cm^{-2}$ in the current study which is also similar to that previously reported in the VL muscle *in vivo* in humans (Maganaris et al., 2001; Reeves et al., 2004b; Erskine et al., 2010). Muscle specific force did not change during DT, as mean VL fascicle force declined by −8% from PT to DT2 which was almost identical (−10%) to the observed mean reduction in npCSA during the same time period. Therefore, the data from the current study suggests that the intrinsic force generating capabilities of the muscles do not change following short term detraining.

Limitations

There are two main limitations in the current study that are important to highlight. First, the estimation of pCSA is derived from an estimate of muscle volume using three axial aCSA measures at 25, 50, and 75% of femur length. The aCSA estimates are themselves made up of composite axial ultrasound scans. Therefore there is the potential to over or underestimate VL muscle volume, and thus pCSA. However, aCSA measures using the technique outlined in the current study have been found to be in almost perfect agreement with MRI measures, with a very small typical error of only 1.7% between the two estimates along the length of the VL muscle (Reeves et al., 2004a). Furthermore, when multiple ultrasound axial aCSA scans are combined to estimate VL muscle volume, there are no significant differences between the derived muscle volumes of ultrasound and MRI, with a 2.2% mean difference between estimates, which was deemed not clinically relevant (Walton et al., 1997). Furthermore, Morse et al. (2007) demonstrated that just a single aCSA axial scan of the quadriceps at either 40, 50, or 60% of femur length correlates significantly and highly (R = 0.84, 0.93, and 0.90, respectively) with the observed quadriceps volume as measured by MRI. In fact, a single scan at 50 or 60% femur length is associated with standard error of the estimate (SEE) of only 13 and 10%, respectively. As such, we would argue that taking three axial scans along the femur length leads to an estimated VL volume with sufficient accuracy, particularly as the pCSA calculated that include such error estimates fall comfortably within the relative changes in pCSA in our results. Lastly, Morse et al. (2007) reported a mean ± S.D. VL muscle volume of 702 ± 108 cm^3 in a group of 18 young recreationally active men. The mean ± S.D. estimated VL volume at baseline of the current study's males within the training group (n = 8) was 741 ± 102 cm^3, and therefore indicates that our current baseline measure of muscle volume are comparable to that of a similar homogenous population. The second limitation in the assessment of muscle architecture with particular reference to fascicle length, was the use of a 4 cm probe as the entire muscle fascicle cannot be recorded in one image. Furthermore, due to the 2D image, the fascicle length may be underestimated when the digitized fascicles do not lie in the image plane. Therefore, future studies investigating this may employ a probe with a scanning width of 6 or 10 cm, or even dual probe approach due to the relative length of VL fascicles (Brennan et al., 2017; Franchi et al., 2018). Finally, a further limitation was the use of a notch filter in the EMG recording system, to remove mains hum, which would have removed the physiological signal at this frequency.

CONCLUSION

Following a prolonged period of RT in young healthy individuals, muscle mass and strength were increased although there were no effects on the specific force of muscle or on systemic levels of TNFα. Furthermore, following a period of short-term detraining, muscle mass and strength were significantly reduced with muscle mass remaining significantly above baseline values following the conclusion of the detraining period. Changes in TNFα were significantly correlated with the reductions in muscle mass following detraining compared to post-training. TNFα may play a part in the detraining induced loss of muscle mass following a period of RT. However, further work is needed to corroborate this and the mechanisms by which this may occur are currently unknown.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Manchester Metropolitan University Cheshire Campus ethics committee with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Manchester Metropolitan University Cheshire Campus ethics committee.

AUTHOR CONTRIBUTIONS

GM, CM, KW, AB, and GO conceived and planned the experiments, supervised the training sessions and contributed to the final version of the manuscript. GM with the support of GO performed all the experiments, statistical analyses, and wrote the manuscript.

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Evidence of a Uniform Muscle-Tendon Unit Adaptation in Healthy Elite Track and Field Jumpers: A Cross Sectional Investigation

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Different adaptive responses to mechanical loading between muscle and tendon can lead to non-uniform biomechanical properties within the muscle-tendon unit. The current study aimed to analyze the mechanical properties of the triceps surae muscle-tendon unit in healthy male and female elite track and field jumpers in order to detect possible inter-limb differences and intra-limb non-uniformities in muscle and tendon adaptation. The triceps surae muscle strength and tendon stiffness were analyzed in both limbs during maximal voluntary isometric plantar flexion contractions using synchronous dynamometry and ultrasonography in sixty-seven healthy young male (n = 35) and female (n = 32) elite international level track and field jumpers (high jump, long jump, triple jump, pole vault). Triceps surae muscle-tendon unit intra-limb uniformity was assessed using between limb symmetry indexes in the muscle strength and tendon stiffness. Independent from sex and jumping discipline the take-off leg showed a significantly higher ($p < 0.05$) triceps surae muscle strength and tendon stiffness, suggesting different habitual mechanical loading between legs. However, despite these inter-limb discrepancies no differences were detected in the symmetry indexes of muscle strength (5.9 ± 9.4%) and tendon stiffness (8.1 ± 11.5%). This was accompanied by a significant correlation between the symmetry indexes of muscle strength and tendon stiffness (r = 0.44; $p < 0.01$; n = 67). Thus, the current findings give evidence for a uniform muscle-tendon unit adaptation in healthy elite track and field jumpers, which can be reflected as a protective mechanism to maintain its integrity to meet the functional demand.

Keywords: triceps surae, muscle strength, tendon stiffness, elite jumpers, athletic training

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INTRODUCTION

The ability to sprint and jump maximally requires high mechanical power outputs and energy generation capabilities at the joints of the lower extremity (Bobbert et al., 1986; Stefanyshyn and Nigg, 1998). Accordingly, the ability to maximize sport performance in jumping and sprint disciplines requires enhanced leg and ankle extensor muscle-tendon unit (MTU) capacities (e.g.,

muscular strength and tendon stiffness), where the muscle and tendon have to be considered as a functional unit to effectively generate high joint moment magnitudes and mechanical power output. Tendons can enhance muscle performance during stretch–shortening cycle activities (e.g., sprinting and jumping) due to their ability to reduce muscular work by storing and releasing elastic energy, which allows muscles to operate at more favorable velocities for force generation (Biewener and Roberts, 2000; Hof et al., 2002). Therefore, it is not surprising that the mechanical and morphological properties of both muscles and tendons have been shown to affect athletic performance (Kubo et al., 1999, 2011; Bojsen-Møller et al., 2005; Stafilidis and Arampatzis, 2007; Albracht and Arampatzis, 2013).

Previous longitudinal investigations demonstrate that increases in muscle strength are commonly accompanied by intermittent changes in tendon stiffness (Kubo et al., 2001, 2012; Arampatzis et al., 2007a,b, 2010; Kongsgaard et al., 2007; Bohm et al., 2014). These modifications in tendon stiffness may serve as a protective mechanism to maintain its integrity to meet the increased functional demand due to muscle strength changes. Nevertheless, recent observations demonstrate differences between muscle and tendon in the responsiveness to mechanical stimuli (Arampatzis et al., 2007a, 2010), time course of the adaptive mechanisms (Kubo et al., 2012) and the effectiveness of tissue turnover (Heinemeier et al., 2013). More specifically, muscles tend to have a superior plasticity in response to a wide range of exercise modalities (Moss et al., 1997; Schoenfeld et al., 2016) in comparison to tendons which seem to respond most effectively to a mechanical stimulus causing high tendon strain magnitudes over a longer time duration (Arampatzis et al., 2007a, 2010; Bohm et al., 2014). These adaptive differences within the MTU may be even more relevant for athletes experiencing high training loads in certain training modalities, especially in athletic disciplines with constant excessive plyometric loading, i.e., in volleyballers, basketballers and track and field jumpers. Plyometric training is, by its nature, a form of high magnitude mechanical loading (likely higher than in any resistance exercise), however, the time durations under which the forces cause tendon strain are rather short with ground contact times mostly under 200 ms during jumping. This could possibly explain why different plyometric loading regimens have not always demonstrated that rapid muscle strength adaptations (Saez de Villarreal et al., 2010) are accompanied by similar adaptive responses in tendons (Burgess et al., 2007; Kubo et al., 2007; Fouré et al., 2009, 2010; Houghton et al., 2013; Bohm et al., 2014; Hirayama et al., 2017). From a biomechanical perspective, an increase in muscle strength in the absence of compensatory-adaptive changes in tendon mechanical properties would place the tendon under a higher internal mechanical demand (i.e., higher strain), hence lead to a non-uniform adaptation within the MTU. Indeed, recent studies show that in adolescent athletes a two-fold stimulus of maturation in association with frequent and predominantly plyometric mechanical loading can lead to fluctuations in muscle strength without accompanying adaptive changes in the tendon (Mersmann et al., 2016). From a practical viewpoint this supports the suggestion that the total jumping volume is a risk factor for tendon overload injuries in elite athletes (Bahr and Bahr, 2014).

In particular, the Achilles tendon (AT) is predisposed for tendon injuries due to a considerably lower safety factor (ratio between ultimate failure stress and functional stress) in comparison to other tendons (Ker et al., 1988; Magnusson et al., 2001) and the prevalence of AT tendinopathies seems to increase after maturity in adult athletes (Cassel et al., 2018; Janssen et al., 2018). A recent study demonstrated higher m. triceps surae (TS) muscle strength and AT stiffness for the take-off leg in comparison to the swing leg in male collegiate track and field jumpers with somewhat higher inter-limb dissimilarities in AT stiffness in relation to muscle strength (Bayliss et al., 2016). Such asymmetrical adaptation could be the result of different habitual loading patterns between the limbs during plyometric exercises and may vary across different track and field jumping events due to differences in the event-specific demand (Deporte and Van Gheluwe, 1989; Perttunen et al., 2000; Plessa et al., 2010; Willwacher et al., 2017). Whether habitual mechanical loading, incorporating these potential discrepancies in various athletic disciplines would induce intra-limb non-uniformities between muscle and tendon adaptation remains unclear in elite jumping event athletes. Taking into account that the habitual mechanical loading may be higher in elite athletes, makes this suggestion plausible, however, there is a lack of sufficient evidence in the literature to suggest clear differences in intra-limb uniformity between elite athletes from different jumping disciplines. Moreover, it is not well-established whether such non-uniformities within the MTU due to habitual loading are potentiated in female elite athletes. Regarding this, it has been demonstrated that females, as well as exhibiting lower muscle strength and tendon stiffness, also show a diminished regulation of muscle and tendon remodeling in response to mechanical loading in comparison to male adults (Magnusson et al., 2007; Miller et al., 2007; Westh et al., 2008; Hansen and Kjær, 2014; McMahon et al., 2018).

Therefore, the aim of this cross-sectional investigation was to examine the inter-limb differences in TS muscle strength and AT stiffness and the intra-limb uniformity within TS MTU using symmetry indexes in healthy male and female elite track and field jumpers. It was hypothesized that in addition to potential leg-specific and sex-related differences in TS muscle strength and AT stiffness, both male and female elite jumping athletes would demonstrate intra-limb non-uniformities between muscle and tendon mechanical properties irrespective of jumping discipline.

MATERIALS AND METHODS

Participants and Experimental Design

As a part of a nationwide study on the TS MTU adaptation, sixty-seven healthy young male (n = 35; age: 23 ± 4 years; body mass: 82 ± 7 kg; body height: 189 ± 7 cm; mean and standard deviation) and female (n = 32; age: 24 ± 4 years; body mass: 63 ± 6 kg; body height: 177 ± 7 cm) elite international level jumping event track and field athletes from the German national team voluntarily participated in the study. The athletes were divided into groups based on their specific athletic event (HJ, high jump; TJ, triple jump; LJ, long jump; PV, pole

vault; **Supplementary Table 1**). Exclusion criteria included any previous AT ruptures and any tendon problems (tendinopathy, etc.) within the last 6 months, which could have potentially influenced the findings. The study was approved by the ethics committee of the German Sport University Cologne and prior to commencing the study all participants gave written informed consent in accordance with the Declaration of Helsinki.

In all athletes the TS MTU mechanical properties (maximal ankle plantar flexion moment and AT stiffness) were assessed in both legs, as a part of a daily training session, at their respective Olympic training centers or at the National Team training camps during or directly prior to the competition period. The preferred jumping leg was defined as the take-off leg, whereas the contralateral non-jumping leg was defined as the swing leg. For triple jumpers the hop leg was considered as the take-off leg. TS MTU intra-limb uniformity was assessed using between limb symmetry indexes of maximal ankle plantar flexion moment and AT stiffness.

Analysis of Triceps Surae Muscle Strength and Achilles Tendon Stiffness

The maximal ankle plantar flexion moment and AT stiffness were assessed in all participants using synchronous ultrasonography and dynamometry on a custom-made device. The analysis methods for TS MTU properties have been described in more detail in a previous study (Ackermans et al., 2016). Briefly, the participants were seated with their ankle and knee joints fixed at 90° angle (thigh and foot perpendicular to the shank) and their foot on a custom-made strain gauge type dynamometer (1000 Hz; TEMULAB®, Protendon GmbH & Co. KG, Aachen, Germany). Each participant's foot was positioned by setting the midpoint of the malleolus lateralis in line with the dynamometer's axis of rotation using the aid of a laser-guided electrical potentiometer system (Ackermans et al., 2016). Prior to the measurements, and in order to "precondition" the tendon, each athlete performed their individualized warm-up routine, followed by a standardized warm-up program of 2–3 min of submaximal and three maximal isometric contractions (Maganaris, 2003). All warm-up contractions were guided by the TEMULAB software.

In order to examine the maximal ankle plantar flexion moment and the force–elongation relationship of the tendon during the loading phase, all athletes performed isometric plantar flexion contractions at different force levels consisting of: three maximal voluntary ankle plantar flexion contractions (MVC) with verbal encouragement, followed by three sustained contractions at 30, 50, and 80% of the maximal joint moment determined during the MVC measurements. The individual maximal ankle plantarflexor muscle strength (TS muscle strength) was normalized to the athlete's body mass (Nm/kg) to ensure an appropriate comparison between subject groups. During all sustained contractions the participants were guided by a visual feedback of the produced joint moment on a computer screen. The resultant joint moments acting about the ankle joint were calculated using inverse dynamics, accounting for the gravitational moments using a prior passive measurement (relaxed muscle in the fixed position). By aligning the axis of rotation of the ankle with the dynamometer's axis of rotation, the ankle joint moment could be considered equal to the moment of the force plate (Ackermans et al., 2016). It is important to note, that the resultant ankle plantar flexion moment is an approximation of the moment produced by the TS muscle, because it does not account for the individual moment contributions of the other synergistic agonist muscles or the antagonist dorsiflexors. The AT force was calculated by dividing the resultant ankle joint moment by the tendon moment arm obtained from the literature (Maganaris et al., 1998).

Following the preconditioning of the tendon, a laser-guided electrical potentiometer system recording linear displacement was used to determine the AT resting length as the distance between the most proximal point of the tuber calcanei and the myotendinous junction (MTJ) of the m. gastrocnemius medialis (both defined using ultrasonography). The displacement of the MTJ of the m. gastrocnemius medialis during the contractions was analyzed using a securely positioned 40 mm linear array ultrasound probe (27 Hz; MyLab^{TM}One, Esaote; Genoa, Italy) and by manually digitizing MTJ in the TEMULAB software (Protendon GmbH & Co. KG, Aachen, Germany). A casing with adjustable straps was used to fix the ultrasound probe on the shank above the MTJ to prevent any movement in relation to the skin. The MTJ displacement was digitized at rest (0% MVC) and at the three sustained contractions (target joint moment held by the participant for 3 s) at the predetermined target ankle joint moment levels (30, 50, and 80% MVC). A specific trial was repeated, when the athlete failed to hold a steady state of 3 s at a range of ±5% of the target joint moment. The tendon elongation at maximal ankle joint moment (100%) was calculated via a linear extrapolation of the elongation at 50 and 80% target joint moments (Ackermans et al., 2016), because instructions (given loading rate and holding the force a certain level) during the maximal plantar flexion contraction may restrict participants ability to contract maximally. This extrapolated approach may play merely a small role for the determined maximal tendon strain as more than half of the entire elongation is achieved within the first 25% of the MVC and consequently only quite small tendon length changes occur between 80 and 100% of the MVC. The reason for using sustained contractions in determining the tendon elongation was to account for potential effects of the tendon viscoelasticity (loading rate dependency) on the force-elongation relationship of the human AT in vivo. For example if a set time (e.g., 2 s) is given to reach maximum force, the absolute loading rate will differ between participants or legs of different strengths. The theoretical consideration of the current approach is that the sustained method may negate loading rate dependency as it accounts for the phase shift due to the time-dependent viscous properties of the viscoelastic material (Meyers and Chawla, 1999). Regarding this issue, in a recent study (McCrum et al., 2018) loading rate effects up to 25% of the MVC were seen during plantar flexion contractions, which were reduced by the sustained method. The day-to-day reliability of this method to assess AT stiffness has been proven previously (Ackermans et al., 2016), by showing no significant differences between trials and

with the mean of the individual ratios of tendon stiffness between days laying close to 1. To account for the effect of an inevitable ankle joint angular rotation on the measured tendon elongation during each contraction (Muramatsu et al., 2001), the changes in ankle joint angle were calculated via 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 (Ackermans et al., 2016). The AT stiffness was calculated as the ratio between the change in the calculated tendon force and change in the resultant tendon elongation between 30 and 80% of maximum tendon force. For more detailed information, including potential methodological drawbacks of the TS MTU analysis and the validity of using sustained contractions for assessing tendon stiffness, please see supplement material in Ackermans et al. (2016) and McCrum et al. (2018).

Analysis of Inter-Limb Symmetry Within the TS MTU

As mentioned above, improvements in muscle strength (increased functional demand on the tendon) are commonly accompanied by intermittent changes in tendon stiffness. In order to examine whether a long-term habitual athletic training leads to a uniform adaptation in the TS muscle strength and AT stiffness between the take-off and swing leg, the symmetry index (SI) was determined (Robinson et al., 1987) between the limbs as follows:

$$SI = \frac{X_{TakeoffLeg} - X_{SwingLeg}}{\frac{1}{2}\left(X_{TakeoffLeg} + X_{SwingLeg}\right)} \times 100\% \quad (1)$$

where $X_{TakeoffLeg}$ is the parameter from the take-off leg and $X_{SwingLeg}$ the corresponding parameter from the swing leg. Accordingly, a positive symmetry index means that the selected parameter has a greater value in take-off leg than swing leg, and a negative symmetry index means that the value is higher in swing leg. Similar SI of TS muscle strength and AT stiffness would illustrate a uniform adaptation within TS MTU (intra-limb uniformity), whereas differences in SIs would indicate to a non-uniform adaptation between muscle and tendon in either take-off or swing leg.

Statistics

The Shapiro-Wilk and Levene's test was used to confirm the normality of distribution and homogeneity of variance of the data ($p > 0.05$). A three-way repeated measures analysis of variance (ANOVA) with leg (take-off vs. swing leg) and jumping discipline (high jump, triple jump, long jump, pole vault) and sex (male vs. female) as factors was performed to examine potential differences between subject groups and legs in TS muscle strength, AT stiffness and maximal AT strain. Potential differences between the SIs were analyzed using an additional three-way ANOVA [sex and discipline as factors and SI of TS muscle strength (SI_{Moment}) and SI of AT stiffness ($SI_{Stiffness}$) as parameters]. In order to identify possible differences in age, body mass, and body height a two-way ANOVA was implemented with sex and jumping discipline as factors. In the case of a detected significant main effect or interaction a Bonferroni *post hoc* comparison was performed. A Pearson product-moment correlation coefficient was used to examine the relationship between TS muscle strength and AT stiffness in the take-off and swing leg separately for male and female jumpers as well as the relationship between the symmetry indexes (SI_{Moment} and $SI_{Stiffness}$) using all analyzed elite jumpers. The level of significance was set at $\alpha = 0.05$ and all statistical analyses were performed using Statistica (Release 10.0; StatSoft Inc., Tulsa, OK, United States). All results in the text and figures are provided as means and standard deviation (SD).

RESULTS

TS MTU Mechanical Properties in Elite Track Field Jumpers

The implemented three-way repeated measures ANOVA showed a significant leg effect ($p < 0.001$) in TS muscle strength with higher values for the take-off in comparison to swing leg independent of jumping discipline (**Figure 1**; no significant interaction). There was a jumping discipline effect ($p = 0.006$), with pole vaulters (PV) showing significantly lower TS muscle strength in comparison to high (HJ; $p = 0.001$), long (LJ; $p = 0.009$) and triple jumpers (TJ; $p = 0.028$) irrespective of athletes' sex and analyzed leg (**Figure 1**). Furthermore, male jumpers showed significantly ($p = 0.002$) higher TS muscle strength compared to female jumpers (**Figure 1**).

For the AT stiffness, the ANOVA revealed a significant leg effect ($p < 0.001$) with greater AT stiffness for the take-off leg in

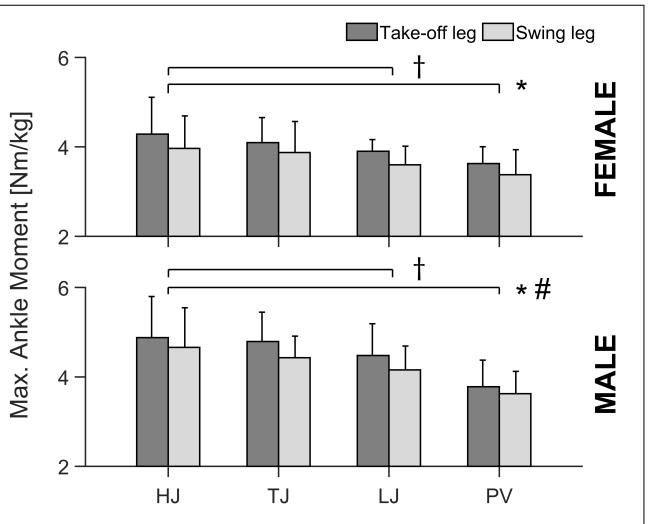

FIGURE 1 | Maximal ankle plantar flexion moment (Max. Ankle Moment) of the take-off and swing leg in all analyzed specific jumping event groups (HJ, high jump; TJ, triple jump; LJ, long jump; PV, pole vault) for both male and female elite athletes. All values are expressed as means with SD (error bars). *Statistically significant difference between take-off and swing leg ($p < 0.05$). #Statistically significant difference between male and female elite athletes ($p < 0.05$). †Statistically significant difference to PV ($p < 0.05$).

comparison to swing leg, irrespective of the jumping discipline and sex (**Figure 2**). Similar to TS muscle strength, there was a jumping discipline effect (p = 0.037) in AT stiffness with lower values for the PV in comparison to all other jumping disciplines (p = 0.038, p = 0.035, and p = 0.041, respectively, for HJ, LJ, and TJ), irrespective of athletes' sex and analyzed leg (**Figure 2**). Moreover, male in comparison to female jumpers showed significantly ($p < 0.001$) higher AT stiffness (**Figure 2**). There were no significant main effects or interactions in maximal AT strain (average values across all disciplines and sex: take-off leg 4.6 ± 1.0 vs. swing leg 4.8 ± 1.1%). Statistically significant correlations ($p < 0.01$) were detected between TS muscle strength and AT stiffness in both male (n = 35; $0.44 \leq r \leq 0.45$) and female (n = 32; $0.51 \leq r \leq 0.58$) jumpers irrespective of the analyzed leg (**Figure 3**).

Inter-Limb Symmetry in TS MTU Mechanical Properties in Elite Track Field Jumpers

The implemented ANOVA revealed no significant differences between SI_{Moment} and $SI_{Stiffness}$ independent of sex and specific athletic discipline (**Figure 4**). The SI of TS muscle strength (SI_{Moment}) values over all the analyzed elite jumpers ranged from −15.8 to 28.5% with an average value of 5.9 ± 9.4% (positive values indicate take-off leg dominance; **Figure 4**). For the SI of AT stiffness ($SI_{Stiffness}$) values in elite jumpers ranged from −15.4 to 36.6% with an average value of 8.1 ± 11.5% (positive values indicate higher stiffness for the take-off leg; **Figure 4**). Statistically significant correlations (r = 0.44; $p < 0.01$; n = 67) were identified between SI_{Moment} and $SI_{Stiffness}$ in both male and female jumpers (**Figure 5**).

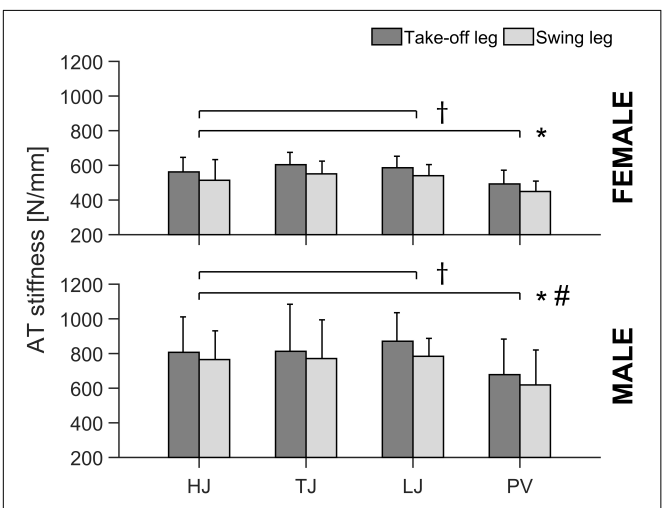

FIGURE 2 | Achilles tendon (AT) stiffness of the take-off and swing leg in all analyzed specific jumping event groups (HJ, high jump; TJ, triple jump; LJ, long jump; PV, pole vault) for both male and female elite athletes. All values are expressed as means with SD (error bars). *Statistically significant difference between take-off and swing leg ($p < 0.05$). #Statistically significant difference between male and female elite athletes ($p < 0.05$). †Statistically significant difference to PV ($p < 0.05$).

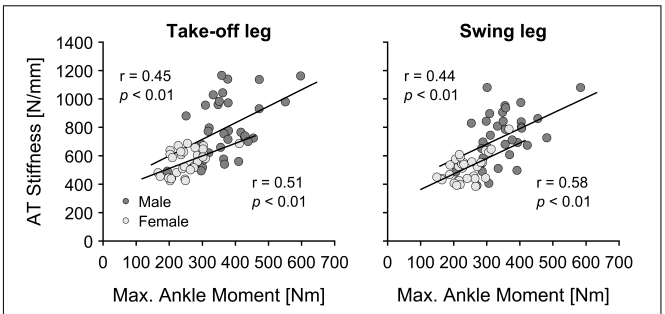

FIGURE 3 | Correlations between maximal ankle plantar flexion moment (Max. Ankle Moment) and Achilles tendon (AT) stiffness in the take-off and swing leg in male (n = 35) and female (n = 32) elite athletes. Correlation coefficients for male elite jumpers (dark gray circles) are shown in the upper left corner and for female elite jumpers (light gray circles) in the lower right corner of the figure.

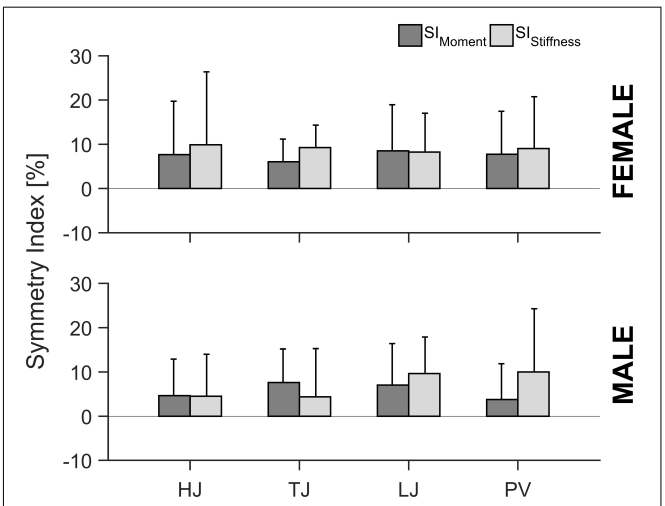

FIGURE 4 | Symmetry index of the maximal ankle plantar flexion moment (SI_{Moment}) and Achilles tendon stiffness ($SI_{Stiffness}$) in all analyzed specific jumping event groups (HJ, high jump; TJ, triple jump; LJ, long jump; PV, pole vault) for both male and female elite athletes. All values are expressed as means with SD (error bars). A positive symmetry index displays a greater value for the take-off leg and a negative symmetry index for the swing leg.

DISCUSSION

This study investigated TS MTU mechanical properties in both legs of healthy male and female elite track and field jumpers to detect possible inter-limb differences and intra-limb non-uniformities in TS muscle strength and AT stiffness due to habitual athletic training. It was hypothesized that elite jumpers will display possible intra-limb non-uniformities between TS muscle strength and tendon stiffness along with potential leg-specific differences in muscle and tendon mechanical properties. However, the hypothesis could not be confirmed, as the leg-specific differences were not accompanied by intra-limb non-uniformities within the MTU, irrespective of jumping event and sex.

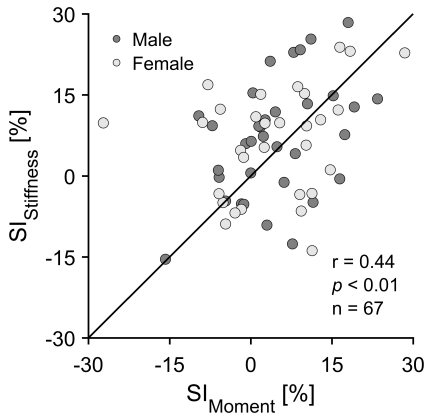

FIGURE 5 | Correlation between the symmetry index of the maximal ankle plantar flexion moment (SI_{Moment}) and Achilles tendon stiffness ($SI_{Stiffness}$) in all analyzed elite athletes. Correlation coefficients were comparable for male elite jumpers (dark gray circles; $r = 0.44$; $p < 0.01$; $n = 35$) and female jumpers (light gray circles; $r = 0.44$; $p = 0.01$; $n = 32$). A positive symmetry index displays a greater value for the take-off leg and a negative symmetry index for the swing leg.

Despite resistance exercise interventions inducing high tendon strains frequently reporting concurrent changes in tendon biomechanical properties along with increased muscle strength (Kubo et al., 2001; Arampatzis et al., 2007a; Kongsgaard et al., 2007), purely plyometric exercise interventions show inconsistent results for tendon adaptation in comparison to skeletal muscle (Burgess et al., 2007; Kubo et al., 2007; Fouré et al., 2009, 2010; de Villarreal et al., 2010; Houghton et al., 2013; Bohm et al., 2014; Hirayama et al., 2017). A reasonable explanation for this could be that tendons may not respond as effectively to loading profiles characterized by short tendon strain durations (Bohm et al., 2014), i.e., plyometric exercises such as repetitive jumping. Nevertheless, the findings of the current study detected a greater AT stiffness (on average ∼9%) along with higher TS muscle strength (∼7%) for the take-off leg in comparison to the swing leg in elite track and field jumpers. Accordingly, the current cross-sectional investigation does not give evidence to suggest that habitual loading in elite jumpers leads to a clear non-uniform adaptation within the MTU as previously shown in adolescent athletes (Mersmann et al., 2016). This is further reflected by the significant correlation between TS muscle strength and tendon stiffness for both male and female elite jumpers (**Figure 3**). Our identified leg-specific differences may relate to a possibly greater total unilateral jumping volume and hence an overall increased mechanical loading of the take-off leg during the training and competition. Similar asymmetric adaptations in muscle and tendon mechanical properties due to habitual loading have been identified also in other sport disciplines (e.g., badminton, fencing, handball) (Couppé et al., 2008; Hansen et al., 2013) and are in line with observations from collegiate-level jumping athletes (Bayliss et al., 2016).

Such a concurrent between limb increase in tendon stiffness and muscle strength in elite athletes may be considered as a protective mechanism to sustain tendon integrity in order to withstand the functional demand from the muscle (Kubo et al., 2001; Arampatzis et al., 2007a; Kongsgaard et al., 2007). Moreover, when considering the relative differences in MTU mechanical properties, we found comparable SIs in muscle strength and tendon stiffness. These findings were irrespective of the athletic discipline or sex (**Figure 4**), indicating that the overall cumulative habitual loading in elite jumping event athletes does not lead to intra-limb non-uniform adaptation within the TS MTU. This suggestion is further supported by the similar maximal AT strain values between take-off and swing leg and the significant correlations between SI_{Moment} and $SI_{Stiffness}$ (**Figure 5**). Moreover, the above findings were independent of sex, indicating the possible different regulation of muscle and tendon remodeling in response to mechanical loading (Magnusson et al., 2007; Miller et al., 2007; Westh et al., 2008; Hansen and Kjær, 2014; McMahon et al., 2018) seems not to affect the uniform development of the TS MTU in female elite athletes. However, one might suggest that our reported inter-limb differences in elite athletes might be a result of biological variation and not due to habitual mechanical loading. Regarding this, it is important to note that in an additional study we analyzed a group of elite international and national level male sprinters (100 m personal best in a range from 10.15 to 10.89 s; mean 10.64 ± 0.23 s) and detected clearly lower intra-limb non-uniformities (on average 0.1 ± 12.3 and 3.3 ± 8.9%, respectively, for the SI_{Moment} and $SI_{Stiffness}$, with take-off leg defined as the front limb in the block start). Based on the current findings we conclude that elite track and field jumpers experienced different habitual mechanical loading profiles between limbs during training and competitions that leads to higher TS muscle strength and AT stiffness for the take-off leg in comparison to the swing leg. However, the magnitude of differences seems not to cause any measurable non-uniform adaptation within the TS MTU regardless of athletic discipline and sex.

From a biomechanical perspective, when increases in muscle strength are not accompanied by increases in tendon stiffness, an imbalance within the muscle-tendon unit may develop, described by a raise in the mechanical demand for the tendon, possibly making it more susceptible for injuries. Opposite to variations in ultimate stress (i.e., stress at tendon failure) between different tendons, ultimate tendon strain is shown to be rather constant (LaCroix et al., 2013). Accordingly, based on previous experimental data from cadaver studies and animal models (Wren et al., 2003; LaCroix et al., 2013), habitual tendon strain can be considered as a central indicator for tendon mechanical demand and the risk of injury. Thus, a uniform adaptation in both muscular strength as well as in tendon stiffness needs to be assured to maintain tendon homeostasis, which may be of particular relevance for the AT due to its low safety factor. Although in the current study we were not able to identify any non-uniformities within the TS MTU, it is important to mention that the measurements of the examined elite track and field athletes were taken in a specific time period (during or directly

prior of the competition period). Therefore, we cannot answer whether there are potential fluctuations in maximal ankle plantar flexion moment and AT stiffness throughout the athletic training over time in elite athletes that would affect our inter- and intra-limb comparison. In relation to this, it is important to note that both male and female jumpers showed significant, albeit moderate correlation coefficients between TS muscle strength and AT stiffness as well as SI_{Moment} vs. $SI_{Stiffness}$ ($0.44 \leq r \leq 0.58$; **Figures 3**, **5**). These findings may indicate that intra- and inter-limb fluctuations in muscle strength during athletic training may not always be accompanied by similar adaptive changes in tendon stiffness, hence potential discordances in muscle and tendon adaptation cannot be excluded in some elite athletes. The above suggestion is supported by the observation that the current pool of elite athletes showed relatively high inter-subject variations in the symmetry indexes and are in line with earlier reports of large individual leg-differences in muscle architecture of m. gastrocnemius medialis in national level jumpers (Aeles et al., 2017). Accordingly, based on the above provided biomechanical perspective for an increased risk of tendon injuries, future studies should investigate longitudinal adaptations of the TS MTU mechanical properties in order to examine whether elite jumpers show similar fluctuations in muscle strength capacities without parallel or compensating changes in tendon stiffness as previously reported in adolescence athletes (Mersmann et al., 2016). This would provide an important information for athletes-coaches and their support teams about the continuous adaptation processes during various phases of athletic training and whether the uniformity within MTU is disrupted.

One might argue that the knee joint being flexed at 90° could place the gastrocnemius muscle in a less favorable position to generate force, which may result in differences of tendon deformation between its subparts, hence influencing the calculated tendon stiffness. The main reasoning to use this joint angle setup was to reduce the inevitable ankle joint angular rotation in comparison to fully-extended knee joint angle (Ackermans et al., 2016) as it leads to a considerable overestimation of the tendon elongation due to the exerted force during the plantar flexion contraction. In a previous study (Ackermans et al., 2016) we used in addition a more dorsiflexed ankle joint position (85° joint angle) with the same knee joint configuration to improve the force potential of the gastrocnemii and their contribution to the net joint moment. Although, we found significantly higher ankle joint moments and tendon elongation for the more dorsiflexed position, there were no significant differences in tendon stiffness between the two setups (Ackermans et al., 2016 Supplemental Data). Therefore, even though a flexed knee joint may change the complex interactions between different subparts of the AT, it seems not to lead to changes in the force-length relationship of the m. gastrocnemius medialis tendon during loading, especially in its "linear part" where the tendon stiffness was calculated. Finally, moment arms from the previous literature were used to calculate the AT force (Maganaris et al., 1998) instead of assessing individual moment arms, which could influence the current AT stiffness values in absolute terms. However, while this may affect the comparison between male and female athletes we believe that this drawback may not significantly affect the main outcomes measures of the current study in terms of inter-limb symmetries and intra-limb uniformity as AT moment arms have been previously shown to be symmetrical between limbs (Bohm et al., 2015).

CONCLUSION

In conclusion, the current findings demonstrate a higher TS muscle strength and greater AT stiffness in the take-off in comparison to swing leg in healthy male and female elite track and field jumpers. Moreover, these inter-limb differences were independent of jumping discipline and athlete sex, suggesting that the limbs of elite jumpers experience different habitual mechanical loading profiles during training and competition. However, the magnitude of differences seems not to cause any measurable non-uniform adaptation within the TS MTU, which can be reflected as a protective mechanism to maintain its integrity to meet the functional demand.

ETHICS STATEMENT

The study was approved by the ethics committee of the German Sport University Cologne and prior to commencing the study all participants gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

GE, FS, and KK participated in the conception and design of the research. GE, FS, and MK performed the experiments. GE, SH, and KK analyzed the data. GE, SH, MK, FS, and KK interpreted results of experiments. GE and KK prepared the figures and drafted manuscript. GE, SH, MK, FS, and KK edited and revised the manuscript and approved final version of manuscript.

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SUPPLEMENTARY MATERIAL

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frontiers in Physiology

ORIGINAL RESEARCH
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Triceps Surae Muscle-Tendon Unit Properties in Preadolescent Children: A Comparison of Artistic Gymnastic Athletes and Non-athletes

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Knowledge regarding the effects of athletic training on the properties of muscle and tendon in preadolescent children is scarce. The current study compared Achilles tendon stiffness, plantar flexor muscle strength and vertical jumping performance of preadolescent athletes and non-athletes to provide insight into the potential effects of systematic athletic training. Twenty-one preadolescent artistic gymnastic athletes (9.2 ± 1.6 years, 15 girls) and 11 similar-aged non-athlete controls (9.0 ± 1.7 years, 6 girls) participated in the study. The training intensity and volume of the athletes was documented for the last 6 months before the measurements. Subsequently, vertical ground reaction forces were measured with a force plate to assess jumping performance during squat (SJ) and countermovement jumps (CMJ) in both groups. Muscle strength of the plantar flexor muscles and Achilles tendon stiffness were examined using ultrasound, electromyography, and dynamometry. The athletes trained 6 days per week with a total of 20 h of training per week. Athletes generated significantly greater plantar flexion moments normalized to body mass compared to non-athletes (1.75 ± 0.32 Nm/kg vs. 1.31 ± 0.33 Nm/kg; $p = 0.001$) and achieved a significantly greater jump height in both types of jumps (21.2 ± 3.62 cm vs. 14.9 ± 2.32 cm; $p < 0.001$ in SJ and 23.4 ± 4.1 cm vs. 16.4 ± 4.1 cm; $p < 0.001$ in CMJ). Achilles tendon stiffness did not show any statistically significant differences ($p = 0.413$) between athletes (116.3 ± 32.5 N/mm) and non-athletes (106.4 ± 32.8 N/mm). Athletes were more likely to reach strain magnitudes close to or higher than 8.5% strain compared to non-athletes (frequency: 24% vs. 9%) indicating an increased mechanical demand for the tendon.

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Although normalized muscle strength and jumping performance were greater in athletes, gymnastic-specific training in preadolescence did not cause a significant adaptation of Achilles tendon stiffness. The potential contribution of the high mechanical demand for the tendon to the increasing risk of tendon overuse call for the implementation of specific exercises in the athletic training of preadolescent athletes that increase tendon stiffness and support a balanced adaptation within the muscle-tendon unit.

Keywords: preadolescent athletes, tendon stiffness, muscle strength, jumping performance, training documentation

INTRODUCTION

Imbalances within muscle-tendon units affect the tendon safety factor [i.e., ratio of tendon ultimate strain to functional operating tendon strain (Ker et al., 1988)], because the ultimate strain of tendons cannot be significantly changed (LaCroix et al., 2013). Thus, in individuals with higher muscle strength, the margin of tolerated mechanical loading is commonly increased by means of greater stiffness of their tendons (Arampatzis et al., 2007b). A higher tendon stiffness will result in less tendon strain at a given tendon force, which might imply less damage, because the strain is the primary mechanical parameter governing tendon damage accumulation for both static and cyclic loading (Wren et al., 2003). However, the types of loading that trigger adaptation and the time course of adaptive changes are different between muscles and tendons, and therefore periods of imbalances in muscle and tendon properties can occur during training (Mersmann et al., 2016). The muscle has a higher metabolic rate compared with the tendon (Laitinen, 1967) and it is likely that muscles adapt at a higher rate to altered mechanical loading. For example, changes in the morphology and architecture of the muscle have been reported after 3 to 4 weeks of heavy resistance training (Seynnes et al., 2007; DeFreitas et al., 2011), while no such fast changes were observed in the mechanical or morphological tendon properties (Mersmann et al., 2017a). In adolescent athletes, it has been shown that an imbalance between muscle strength and tendon stiffness results in a higher mechanical demand for the tendon compared to non-athlete controls (Mersmann et al., 2017a).

Research of the last 15 years has shown that the mechanical properties of tendons are crucial for muscle-tendon unit functioning during walking (Lichtwark et al., 2007; Lai et al., 2015), running (Arampatzis et al., 2006; Albracht and Arampatzis, 2013; Lai et al., 2018), and jumping (Bojsen-Møller et al., 2005; Ishikawa and Komi, 2008; Nikolaidou et al., 2017). These studies have shown that the elasticity of tendons enables the storage and release of strain energy during movements, which facilitates the operating conditions of muscle fascicles with regard to the force-length-velocity relationships. This energy exchange within the muscle-tendon unit seems to be optimal at a given balance of muscle strength and tendon stiffness (Lichtwark et al., 2007; Orselli et al., 2017). Therefore, in healthy adults, muscle strength and tendon stiffness are usually strongly associated (Arampatzis et al., 2007b). Furthermore, an adequate strain applied to the tendon is important and necessary for tendon healthiness and adaptability. In previous studies (Arampatzis et al., 2007a, 2010; Bohm et al., 2014) we demonstrated that cyclic loading of the tendon, which caused tendon strain of approximately 4.5 to 6.5% for about 3 s per cycle, is a very effective stimulus to improve the mechanical, morphological and material properties of the tendon. Mechanical tendon loading that introduces low-level tendon strain ($\leq$3%) on the other hand cannot improve tendon properties (Arampatzis et al., 2007a, 2010). However, tendon strain higher than 9% may overload the tissue, result in degeneration of the tendon and impair its structural integrity (Wang et al., 2013). Strain magnitudes higher than 8.5–9.0% during maximum isometric contractions might be indicative of imbalances within the muscle-tendon unit in terms of tendon stiffness being too low compared to muscle strength (Mersmann et al., 2016; Bohm et al., 2019).

Although training-induced muscle hypertrophy in preadolescent children is still debated (Ramsay et al., 1990; Ozmun et al., 1994; Malina, 2006; Granacher et al., 2011), resistance exercise and athletic training clearly improve muscle strength (Faigenbaum et al., 1999; Granacher et al., 2011; Behm et al., 2017) and jumping performance in preadolescence (Bassa et al., 2012; Marina and Memni, 2014; Moran et al., 2017). Yet, to our knowledge, no study has investigated the effect of athletic training on tendon mechanical properties in preadolescent children. A study by Waugh et al. (2014) found a significant increase in both muscle strength and tendon stiffness in prepubertal children after an exercise intervention of 10 weeks. However, the mechanical stimulus used in that study is known to be effective for tendon adaptation (i.e., high magnitude, 2–3 s duration strain) and might not be representative of the effects of sport-specific loading. In fact, certain types of sport-specific athletic training, at least in adolescent, seems to induce imbalances in the development of muscle strength and tendon stiffness increasing the mechanical demand for the tendon (Mersmann et al., 2016, 2017a). Knowledge about the effects of athletic training on the muscle-tendon properties in preadolescent children is important for the improvement of our understanding with respect to muscle and tendon plasticity in this age.

The main objective of the current study was to compare the Achilles tendon mechanical properties, plantar flexor muscle strength and vertical jumping performance of preadolescent athletes and non-athletes in order to estimate the potential effects of systematic athletic training. We hypothesized higher muscle strength, tendon stiffness and jumping performance in athletes compared to non-athlete controls.

MATERIALS AND METHODS

Participants and Experimental Design

Twenty-one preadolescent artistic gymnastic athletes (6 males, 15 females; ≈20 h of sport-specific training per week) and a control group of 11 untrained participants (5 males, 6 females; ∼3 h of sport activity per week in school and no further sports participation) with an age of about 9 years (**Table 3**) agreed to participate in the study. The study was approved by the university ethics committee of the Democritus University of Thrace, and all participants (and respective legal guardians) signed informed consent to the experimental procedures. The pubertal status of each child was determined by their parents, and the children were classified according to pubic hair, genital and breast development as described by Marshall and Tanner (1969, 1970). Each parent determined the proper stage according to drawings representing the five different Tanner stages. Additionally, information on the skeletal age of the athletes was provided by the parents following a routine medical examination 1 month after our measurements. The diagnosis was done by a pediatric radiologist based on radiographic images obtained from the left hand of the participants. Due to the exposure to radiation, no radiographic images were made from the non-athletes group. The measurements of muscle strength and tendon mechanical properties were conducted on the right leg in every participant. Four females (2 athletes and 2 non-athletes) had a dominant left leg (i.e., leading leg in a forward fall). However, in participants who did not engage in physical activity related to strong unilateral loading, we did not expect significant differences in muscle- strength or tendon stiffness between sides (Bohm et al., 2015).

Training Documentation

We started the training documentation 6 months before the measurements, in order to quantify the training intensity and training volume. The training was documented by one observer with experience in artistic gymnastics (N.P.) for 1 week (six training units) in the middle of each month (second or third week of the month). The training consisted of a warm-up, muscle strength exercises (mostly with body weight), specific exercises of the lower limbs for the improvement of the jump performance, which involved a variety of jumps and acrobatic elements, and finally the main training for each apparatus. All athletes performed the same number of repetitions (exercises) during warm-up, muscle strength and jump exercises. The only difference was present during the main training, as girls trained on one more apparatus for the lower limbs (i.e., balance beam). Based on our assessment, the variation of overall training volume between athletes was approximately ± 5%. A repetition was classified as medium intensity when during the execution the athletes focused more on a proper technique, and high intensity when the athlete had to perform the repetition with maximal effort. Detailed information regarding the training contents is presented in **Table 1**. **Table 2** shows an example of a typical weekly training program including the number of repetitions and duration of the training contents.

Measurement of Jump Performance

After a standardized warm-up including 3 min of running, 10 submaximal jumps and 5 submaximal isometric ankle plantar flexion contractions, each participant performed first three trials of maximum effort countermovement jumps (CMJ) and then three trials of squat jumps (SJ). The CMJ was performed starting from a standing position. The participants were instructed to quickly squat to a knee angle of approximately 90° (checked by visual observation by the investigator) and then to jump immediately as high as possible afterward. For the SJ, the participants were instructed to take the starting position with a knee angle of 90°, hold that position for 3 s, as determined by the experimenter, and then to perform the jump as high as possible without countermovement. In both jumps, the arms were held akimbo. The vertical ground reaction forces (GRF) during the jumps were measured with a force plate sampling at 1,000 Hz (Kistler 9281CA, Winterthur, Switzerland) and the vertical take-off velocity of the center of mass during the jumps was calculated through the integration of the vertical GRF over the time. Mechanical power applied to the center of mass was determined as the product of vertical ground reaction force and vertical center of mass velocity. The propulsion phase is defined in both SJ and CMJ from the start of the upward movement of the center of mass until take-off. The mean mechanical power was calculated by dividing the integral of power from the propulsion phase by propulsion time. Further, we calculated the eccentric utilization ratio (EUR) as the ratio of CMJ height (or mean mechanical power) to SJ height (or mean mechanical power). The trial with the greatest jump height was considered in the statistical analysis.

Measurement of Maximum Ankle Joint Moment

Plantar flexor muscle strength was measured combining dynamometry, kinematic, and electromyography (EMG) recordings. Effects of gravitational forces and the misalignment of rotation axes of ankle joint and dynamometer were considered using inverse dynamics (Arampatzis et al., 2005). For this purpose, seven reflective markers were fixed to the following anatomical landmarks: trochanter major, the most prominent points of the lateral and medial femoral epicondyles, lateral and medial malleolus, the dorsal gap between the distal metaphysis of the 2nd and 3rd metatarsals and tuber calcaneus, as well as five markers fixed on the dynamometer: axis of the dynamometer, lever of the dynamometer, two markers on the foot plate located lateral and medial to the line of force application and one additional to define the surface of the foot plate. Kinematic data were recorded using a Vicon motion capture system (version 1.8.5; Vicon Motion Systems, Oxford, United Kingdom) integrating eight cameras operating at 100 Hz. The electromyographic (EMG) activity of the head of the tibialis anterior was recorded using two bipolar surface electrodes (Blue Sensor N, Ambu GmbH, Bad Nauheim, Germany) fixed over the mid-portion of the muscle belly with an inter-electrode distance of 2 cm after shaving and cleaning the skin. EMG data was captured at 1,000 Hz (Myon m320RX; Myon, Baar, Switzerland) and transmitted to the Vicon system via a 64-channel A-D

TABLE 1 | Detailed description of each exercise for the lower extremities documented for the quantification of the training intensity and volume.

Part of the training	Exercise	Description of the exercise
Warm-up	A-skip	– Skipping forward, alternating lifting one knee to waist-height while keeping the other straight
	Butt kicks	– Alternating flexing the knee and kicking the heel up toward the gluteus, bringing the foot back
	High knees	– Lifting up the knee as high as it will go and raise the opposite arm, switching quickly so the left knee is up before the right foot lands
	Grapevine	– Performing a criss-cross with the legs by stepping side-to-side
	Straight leg shuffle	– Alternating moving the straight leg forward at high cadence
	Backward running	– Pushing off the ground with the balls of the feet while moving backward
Muscle strength	Squat jump	– 1–2 kg ankle weights: Maximum jump following 1–2 s in a squat position
	Squat	– 10–20 kg barbell: Squats with 1–2 s with 1–2 s in squat position
	Calf raises	– Plantar flexion using co-athlete (piggyback) as additional weight
	Plinth jump	– Countermovement jump with arm swing onto and off a plinth of 45 to 70 cm height
	Vertical depth jump	– Drop jump from the top of a plinth of 45 to 70 cm height
Jump performance improvement	Two-Legged hops	– Repetitive two-legged long jumps (also called bunny hops)
	Power skipping	– Fast skipping, lifting the upper leg as high as possible
	Alternate leg bounding	– Running with long strides, placing emphasis on hang time
	Lateral jump	– Lateral jump side to side (modification: with forward displacement)
	Tuck jump	– Forward jump while flexing the hip and knees and in the air to a crouched position
	Pike jump	– Forward jump with dynamic hip flexion in the air while keeping the legs straight
	Straddle jump	– A vertical jump where the legs are lifted into an airborne straddle (90° wide open) with arms and trunk extended over the legs as they are elevated
Main training (apparatus)	Blockings	– In gymnastics term "punch": A rapid bounce off the floor or apparatus that converts horizontal speed to vertical
	Landings	– Landings after jumps, acrobatic exercises and dismounts of the apparatus
	Jumps	– Jumps appearing during the floor exercises and on the balance beam

converter. The participants were asked to perform five isometric maximum voluntary ankle plantar flexion contractions (MVC) on a dynamometer (Cybex 6000, Ronkonkoma, New York, NY, United States) at 70° trunk flexion (supine = 0°) with the knee fully extended (180°) and the ankle joint at 0°, 5°, 10°, 15°, and 20° dorsiflexion (0° = tibia perpendicular to the sole), respectively. If a participant was not able to reach some of these degrees of dorsiflexion, those trials were not performed. All the athletes were able to perform the above mentioned five trials, while the non-athletes were able to reach a maximum of 15° dorsiflexion. A passive ankle plantar- and dorsiflexion trial (driven by the dynamometer at 5°/s) and two trials of dorsiflexion contractions were recorded to account for gravitational forces and to establish an activation-flexion moment relationship that was used to estimate the ankle dorsiflexion moment generated during maximum effort ankle plantar flexion due to antagonistic co-activation (Mademli et al., 2004).

Measurement of Tendon Mechanical Properties

For the assessment of the Achilles tendon mechanical properties, the participants completed five trials of isometric ramp contractions (i.e., increasing effort steadily from rest to maximum in ≈4 s followed by a plateau at maximum effort of 2–3 s) with the ankle joint angle at 0° and rest between ramp contractions of 3 min. The displacement of the myotendinous junction (MTJ) of the gastrocnemius medialis muscle-tendon unit during the contractions was captured at 85 Hz by a 60 mm linear ultrasound probe (7.5 MHz; Chison, Model Q3, Wuxi, China). The ankle plantar flexion moments were calculated using the same approach as for the MVC calculation (i.e., correction for axes misalignments, gravitational forces, and antagonistic co-activation), and the ultrasound images were synchronized offline with the data captured in the Vicon system using an externally induced voltage peak, which could be identified in both the ultrasound and the analog data stream. The effect of unavoidable joint angular rotation on the displacement of MTJ during the ramp contractions was taken into consideration by capturing the displacement of the MTJ during a passive rotation of the ankle joint (Arampatzis et al., 2008). The elongation of the tendon was calculated as the difference of the MTJ displacement

TABLE 2 | Description of the training documentation per week (six training units per week).

Part of the training	Sessions per week	Total repetitions[a]	Duration
Warm-up	6 / 6		180 min
Muscle strength	5 / 6	150 medium intensity 450 high intensity	210 min
Specific exercises for improvement of jump performance	5 / 6	225 medium intensity 225 high intensity	75 min
Main training [apparatus]	6 / 6	480 high intensity	720 min

[a] *Total repetitions: The sum of the plyometric exercises, jumps, blocking, and landing elements; high intensity was documented when the athlete was instructed to perform the exercise with maximal effort (e.g., jump as high/fast as possible); medium intensity was documented when the athlete was instructed to perform the exercise focusing more to technique rather than with maximal effort.*

measured during the active contractions and the passive joint rotation. To measure the resting length of the Achilles tendon, the participants were seated on the dynamometer with fully extended knee and the ankle at −20° (plantar flexion), as in this position the inactive gastrocnemius medialis muscle-tendon unit is slack (De Monte et al., 2006). The length of the curved path from the calcaneal tuberosity to the MTJ was defined as the resting length.

Tendon force was calculated by dividing the ankle plantar flexion moment by the tendon moment arm, which was calculated based on the approach suggested by Fath et al. (2010). In short, the moment arm was estimated based on the relationship of MTJ displacement and ankle angular rotation between 5° dorsiflexion and −10° plantar flexion [i.e., range of negligible passive tendon strain; De Monte et al. (2006)]. The moment arm was adjusted to changes induced by force application based on the data of Maganaris et al. (2000). The displacement of the MTJ during the ramp contractions and passive trials was digitized manually by tracking its position frame by frame using a custom-written MATLAB interface (The MathWorks, version 2012b, United States). The tracking was done by one experienced observer (N.P.) and the force-elongation relationship of the five trials of each participant was averaged to achieve excellent reliability (Schulze et al., 2012). Tendon stiffness was then calculated between 50 and 100% of the peak tendon force of the averaged force-elongation curve. We further calculated the normalized tendon stiffness (i.e., the product of stiffness and rest length) that represents the slope of the force-strain curve.

Statistics

The statistical analyses were conducted in SPSS (version 25.0; IBM, Armonk, NY, United States). Normal distribution of the data was checked for all parameters using the Shapiro-Wilk test. For the non-normally distributed parameters, which were body mass, body mass index, co-activation, and height of the CMJ, we compared the groups using the non-parametric Krustal–Wallis one-way analysis of variance for independent samples. For all other parameters we performed a one-way analysis of covariance (ANCOVA) for the effect of group (i.e., athletes and non-athletes) with sex as covariate. We calculated the effect size (f) for statistically significant observations in G*Power (Version 3.1.6; HHU, Düsseldorf, Germany; Faul et al., 2007) based on the partial eta squared. The level of significance for all comparisons was set to $\alpha = 0.05$. In all figures, the data are presented as mean ± standard error of mean (SEM), whereas in the text and tables they are expressed as mean ± standard deviation (SD).

RESULTS

There were no significant differences of between the two groups with regard to age ($p = 0.906$), body height ($p = 0.339$), mass ($p = 0.812$), body mass index (B.M.I.) ($p = 0.984$), and tibia length ($p = 0.394$; **Table 3**). The majority of the athletes were in Tanner stage I, while more non-athletes were in stage II than in I (**Table 3**). According to the evaluation of the radiographic images of the athletes, their skeletal age was on average 1 year less (1 ± 0.8 yr) compared to the chronological age. The athletes completed a training program of about 20 h per week (over 6 days per week). Based on our assessment regarding the intensity of the training (i.e., low intensity during warm-up, medium to high intensity during the muscle strength and the gymnastic specific training, and high intensity during the main on-apparatus training), we found almost an equal intensity distribution among the training (34% low, 30% medium, and 36% high).

The maximum plantar flexion moments normalized to body mass were significantly greater in the athletes compared to the non-athletes ($f = 0.65$, $p = 0.001$; **Table 4**). However, the non-normalized maximum plantar flexion moments did not show statistically significant differences between athletes and non-athletes ($p = 0.06$; **Table 4**). Athletes showed a significantly lower co-activation than the non-athletes during the maximum plantar flexion contraction ($f = 0.45$, $p = 0.021$; **Table 4**). We found significantly greater jump height in athletes compared to non-athletes in both jumps ($f_{SJ} = 0.94$, $f_{CMJ} = 0.83$, $p < 0.001$ for the SJ and CMJ, respectively; **Figure 1A**), as well as greater mean mechanical power during the propulsion phase ($f = 0.6$, $p = 0.004$ for the SJ and CMJ; **Figure 1B**). The duration of the propulsion phase did not show any significant differences

TABLE 3 | Anthropometric data and Tanner scale of the preadolescent athletes and non-athletes.

	Athletes ($n = 21$)	Non-athletes ($n = 11$)
Age [years]	9.2 ± 1.7	9.0 ± 1.7
Height [cm]	131.1 ± 8.0	134.6 ± 11.7
Body mass [kg]	28.9 ± 6.4	31.1 ± 9.0
Tibia length [cm]	28.7 ± 2.2	29.4 ± 2.7
BMI [kg/m^2]	16.6 ± 2.0	16.9 ± 3.0
Percentage in Tanner stage I	66.6%	45.5%
Percentage in Tanner stage II	33.4%	54.5%

Values are means ± standard deviation (SD). BMI: Body mass index. There were no significant differences between groups in the anthropometric data. Values for the Tanner scale are in percentage of the total participants in each group.

TABLE 4 | Ankle joint moment and jump performance of the preadolescent athletes and non-athletes.

	Athletes ($n = 21$)	Non-athletes ($n = 11$)
Maximum plantar flexion moment [Nm]	50.6 ± 14.6	40.4 ± 14.1
Normalized max. plantar flex. moment [Nm/kg]*	1.75 ± 0.32	1.31 ± 0.33
Antagonistic co-activation [%]*	4.0 ± 2.2	7.1 ± 4.5
Propulsion time during SJ [ms]	316 ± 42	352 ± 71
Propulsion time during CMJ [ms]	260 ± 37	247 ± 61
Eccentric utilization ratio [jump height]	1.10 ± 0.12	1.09 ± 0.19
Eccentric utilization ratio [mean mechanical power/kg]	1.39 ± 0.18	1.50 ± 0.35

*Values are means ± standard deviation. Maximum plantar flexion moment was normalized to body mass. Antagonistic co-activation is the antagonistic moment normalized to the resultant ankle joint moment. SJ: Squat jump, CMJ: Countermovement jump. *Significant difference between athletes and non-athletes, $p < 0.05$.*

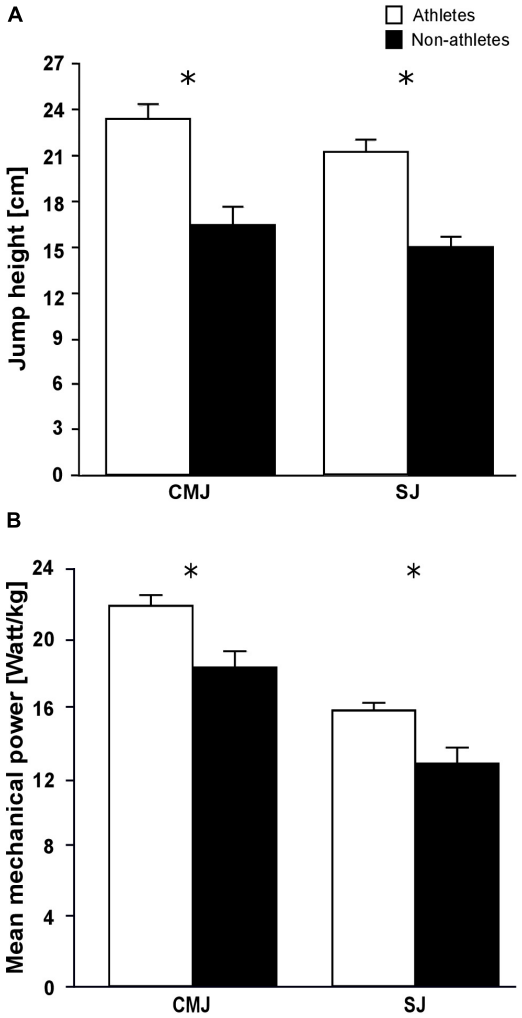

FIGURE 1 | Mean values and standard error (error bars) of **(A)** jump height and **(B)** mean mechanical power during the propulsion phase of preadolescent athletes and non-athletes during the countermovement (CMJ) and squat jump (SJ). *Significant difference between athletes and non-athletes $p < 0.05$.

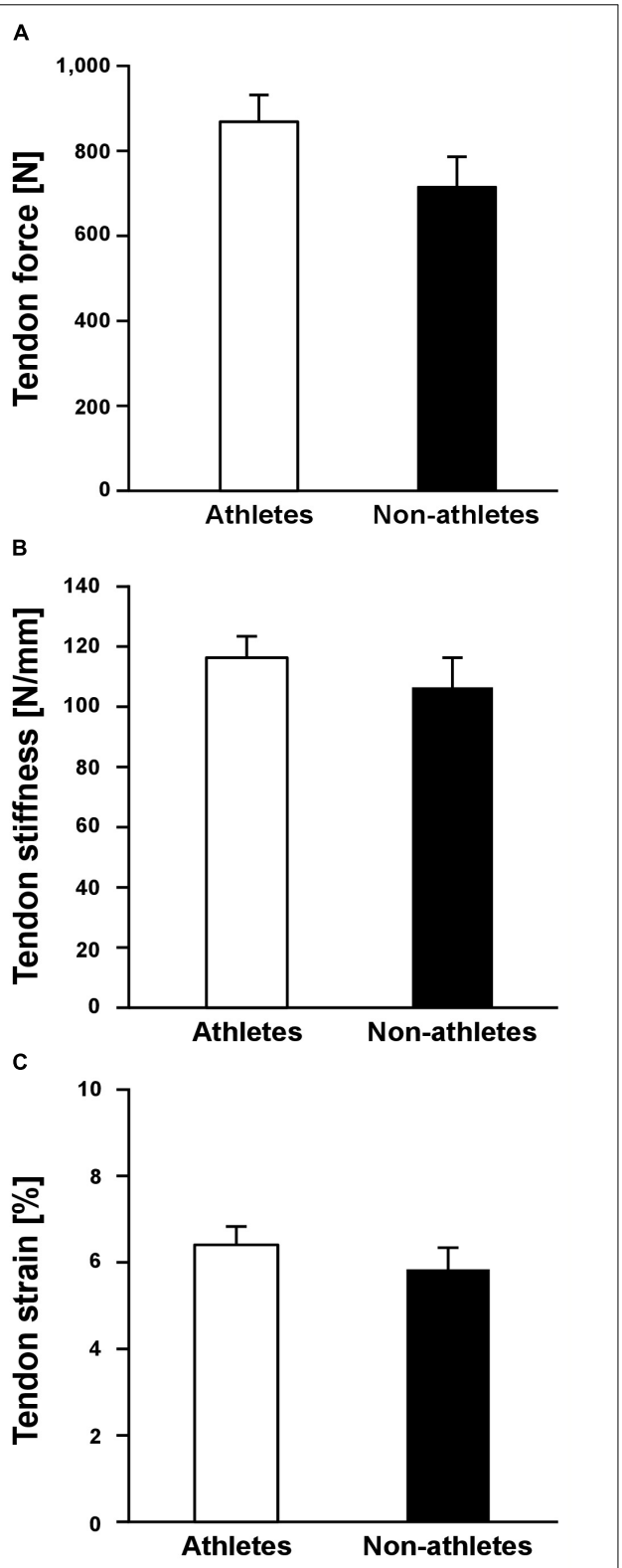

FIGURE 2 | Mean values and standard error (error bars) of Achilles tendon force **(A)**, tendon stiffness **(B)** and tendon strain **(C)** during maximum isometric contractions of preadolescent athletes and non-athletes. No significant differences ($p > 0.05$) were found between the two groups.

between the two groups in both SJ ($p = 0.132$) and CMJ ($p = 0.251$; **Table 4**). The EUR_{height} and EUR_{power} also did not show any significant differences between groups ($p = 0.852$ and $p = 0.209$, respectively; **Table 4**).

Athletes and non-athletes did not show any significant differences in maximum Achilles tendon force ($p = 0.086$; **Figure 2A**), Achilles tendon stiffness ($p = 0.413$; **Figure 2B**), normalized Achilles tendon stiffness ($p = 0.513$; **Table 5**) and maximum Achilles tendon strain ($p = 0.222$; **Figure 2C**). Finally, we did not find any statistically significant differences between the two groups in the Achilles tendon moment arm ($p = 0.587$; **Table 5**), rest length ($p = 0.707$; **Table 5**) and maximum tendon elongation ($p = 0.152$; **Table 5**). However, when examining the individual tendon strain values during the maximum isometric contractions, it is notable that athletes were more likely to reach

TABLE 5 | Achilles tendon properties of the preadolescent athletes and non-athletes.

	Athletes (n = 21)	Non-athletes (n = 11)
Normalized Achilles tendon stiffness [kN/strain]	14.5 ± 5.6	13.1 ± 5.7
Moment arm [mm]	42.2 ± 6.0	40.7 ± 9.1
Rest length [mm]	128.8 ± 22.3	124.3 ± 25.3
Maximum elongation [mm]	7.71 ± 2.1	6.85 ± 1.6

Values are means ± standard deviation. Achilles tendon stiffness was normalized to tendon rest length.

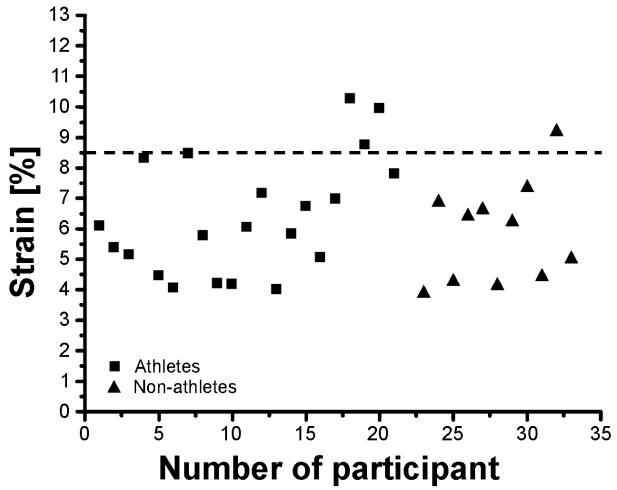

FIGURE 3 | Individual Achilles tendon strain values during maximum isometric contractions in the investigated preadolescent athletes and non-athletes.

strain magnitudes close to or higher than 8.5% strain compared to non-athletes (frequency of 24% in athletes and 9% in non-athletes; **Figure 3**).

DISCUSSION

The present study compared the Achilles tendon mechanical properties, plantar flexor muscle strength and vertical jumping performance of preadolescent athletes and non-athletes to estimate the potential effects of systematic athletic training. We hypothesized higher values in all of these parameters in athletes compared to an age-matched group of non-athletes. We found greater jumping performance and muscle strength normalized to body mass in athletes, but no differences in tendon stiffness between the two groups. Therefore, our results only partly confirmed our hypothesis.

The investigated athletes completed a training program of about 20 h per week (over 6 days per week) with almost 5 h of specific muscle strength and jumping training. Further, the main on-apparatus gymnastic training of about 12 h included several plyometric and landing exercises. These data show that the training volume in the athletes was quite high and could account for an increase in muscle strength. From different studies in the previous years (Faigenbaum et al., 1996; Falk and Eliakim, 2003; Granacher et al., 2011, 2016) it is well accepted that even in childhood, physical exercise (e.g., resistance training, plyometric training) can increase muscle strength. There are several studies (e.g., Faigenbaum et al., 1999; Chaouachi et al., 2014) and reviews (Malina, 2006), which reported increases in muscle strength following plyometric, balance and resistance training in prepubertal children, with strength gains of up to 40% after 8 weeks (Faigenbaum et al., 1999). Also, two meta-analyses of Payne et al. (1997) and Behringer et al. (2010) calculated effect sizes of 0.75 and 1.12, respectively, providing evidence that resistance training increases muscle strength in children. Therefore, our findings of greater normalized ankle plantar flexor strength in athletes compared to non-athletes were not surprising. Considering that non-athletes were only slightly and not statistically significantly heavier compared to the athletes, we expected greater absolute ankle plantar flexor moments for athletes. Surprisingly, the maximum absolute ankle joint moments did not differ significantly between athletes and non-athletes. One explanation could be the lower state of maturity of the athletes, indicated by the higher percentage of athletes in the first Tanner stage than in non-athletes. Further, the radiographic images provided evidence that the athletes were delayed about 1 year in their skeletal development compared to their chronological age. Although the chronological age was similar in the two groups, the state of maturation seemed to be decelerated in the athletes, which is likely to affect the muscle strength development as well. In fact, when accounting for the difference in the maturation status by including the Tanner stage as additional covariate in the ANCOVA, we found significant differences (p = 0.001) in maximum plantar flexor moment between the two groups. Therefore, the missing differences in absolute muscle strength were very likely due to this difference in maturation. Several studies in the past reported a delay of one to 3 years of the skeletal compared to the chronological age in early- and mid-adolescent gymnasts (Novotny and Taftlova, 1971; Beunen et al., 1981; Claessens et al., 1991; Georgopoulos et al., 2004). Irrespective of the generalizability of these findings (see Malina et al., 2013) or the attribution of this delay to mechanical loading, energy balance (due to caloric restriction), selection or a concert of factors (Baxter-Jones et al., 1994; Rogol et al., 2000), which were beyond the scope of our study, our data suggest that a delay in the maturation process can occur in athletic gymnasts even before puberty.

Achilles tendon stiffness did not differ significantly between the two groups, despite the fact that the exercise loading was essentially higher in athletes. In adolescent athletes, Mersmann et al. (2017a) found volleyball athletes have both greater patellar tendon force and stiffness compared to an age-matched control group of non-athletes. Compared to the findings of the current study this indicates that the effects of athletic training on the adaptation of the muscle-tendon properties in preadolescent children and adolescents might be different. Differences in the hormone status between preadolescents and adolescents (Korth-Schutz et al., 1976; Round et al., 1999;

Veldhuis et al., 2000) could provide some explanation for the different effects of training. The concentration of testosterone and oestrogen levels in preadolescence (i.e., Tanner stage I and II) is negligible (Round et al., 1999). However, these concentrations increase significantly after reaching Tanner stage III (Viru et al., 1998, 1999), which promotes the development and increase of muscle mass, physiological muscle cross-sectional area (Richmond and Rogol, 2007; Vingren et al., 2010) and the anabolic response of muscle to training (Hansen and Kjaer, 2014). It is well accepted that maximum muscle strength, or more precisely the force applied to the tendon during maximum isometric contractions, associates to tendon stiffness (Arampatzis et al., 2007b; Seynnes et al., 2009) and is an important mediator for tendon adaptation in both adults (Seynnes et al., 2009) and during childhood development (Waugh et al., 2012). Thus, it seems likely that the absence of marked differences in tendon force between athletes and non-athletes was the reason for the similar Achilles tendon stiffness. The clear differences in tendon force that were present between the respective late-adolescent cohorts in our earlier work (Mersmann et al., 2017a) explain the different results between studies. This lends support to the idea that hormonal changes during puberty promote the responsiveness of muscle to mechanical loading, especially with regard to muscle hypertrophy, which leads to more apparent differences in both muscle strength and tendon stiffness between athletes and untrained counterparts.

The tendon strain during the maximum isometric contractions was not significantly different between athletes and non-athletes in the present study, suggesting that athletic training in preadolescence does not increase the mechanical demand of the tendon as in adolescence (Mersmann et al., 2016), despite comparable mechanical loading during the training process. However, looking at the individual strain values during the maximum isometric contractions, it is notable that athletes were more likely to reach high strain magnitudes, which would indicate a high mechanical demand for the tendon and might indicate imbalances within the muscle-tendon unit. Further, when accounting for the apparent differences in maturation by including the Tanner stage as covariate in the statistical analysis, the differences in tendon stiffness were, in contrast to muscle strength, not significantly different between groups ($p = 0.364$). This also suggests that the athletic activity of the artistic gymnasts might lead to a stronger and/or more consistent adaptation in the plantar flexor muscles compared to its tendon. Thus, although the average tendon strain did not differ between the two groups, at least in some preadolescent athletes a specific training to increase Achilles tendon stiffness is recommendable from a preventive point of view to reduce the mechanical demand for the tendon. Waugh et al. (2014) reported an increase of Achilles tendon stiffness after 10 weeks of strength training in prepubertal children, indicating a possible adaptive potential of tendon mechanical properties in preadolescence. The specific athletic training for the lower extremities in our athletes was dominated by jumping, landing and plyometric exercises, which are the specific loading characteristic in artistic gymnastics. This kind of loading is characterized by high load magnitude and short duration of loading, which results in a high strain rate in the Achilles tendon. Several studies reported a low potential of plyometric training on tendon adaptation despite the high magnitude of tendon loading (Burgess et al., 2007; Kubo et al., 2007; Fouré et al., 2009, 2011; Bohm et al., 2014). Though this is not a consistent finding (Burgess et al., 2007; Wu et al., 2010; Hirayama et al., 2017), all studies directly comparing the effects of plyometric training to training routines that used lower strain rates and longer strain durations consistently show lower adaptive responses in the plyometric training groups (Burgess et al., 2007; Kubo et al., 2007; Bohm et al., 2014). Systematic research of our group provided evidence that an effective training stimulus for tendon adaptation is a combination of high loading magnitude, an appropriate load duration in every repetition (i.e., 3 s) and repetitive loading (Arampatzis et al., 2007a, 2010; Bohm et al., 2014). It can be argued that in some athletes in the present study, the sport-specific loading triggered an increase of muscle strength without sufficiently stimulating the adaptation of the tendon, which resulted in high mechanical demand for the tendinous structures. Therefore, specific tendon training to increase tendon stiffness is, at least in some individuals, recommended also in preadolescent athletes.

In both athletes and non-athletes, the jump height in the CMJ was 10% greater compared to the SJ and the mean mechanical power during the propulsion phase was 38% greater in the athletes and 43% in the non-athletes. Both groups appeared to utilize the stretch-shortening cycle equally to augment jumping performance. Comparable differences in jumping height and mean mechanical power between SJ and CMJ have been reported in adult athletes and non-athletes (Bobbert et al., 1996; Pazin et al., 2013; Nikolaidou et al., 2017), which indicates similar neuromuscular mechanisms for promoting jump height using a countermovement in preadolescent children. The jumping height in both SJ and CMJ was 30% greater in athletes and the mean mechanical power during the propulsion phase was 20% and 16% greater in SJ and CMJ, respectively, compared to the non-athlete controls. Although the contribution of the plantar flexors to the jumping height and mean mechanical power during the propulsion phase is important [i.e., 29 and 13%, respectively, (Nikolaidou et al., 2017)] other muscles within the lower extremities (i.e., knee and hip extensors) also contribute to the jumping performance (Anderson and Pandy, 1993; Nikolaidou et al., 2017). Exercises as for example hopping, lateral, pike and straddle jumps, blocking, and landings were substantial components of the training in the investigated athletes. In average the athletes participating in our study performed on a weekly basis approximately 1,300 repetitions of jumps either with medium or high intensity (medium focusing to proper technique and high with maximal effort), which indicates an increased mechanical load of all muscles within the lower extremities compared to the non-athlete control group. Furthermore, this specific gymnastic training with the large amount of plyometric loading can improve the neuromuscular coordination between the lower extremity muscles (Komi, 2000; Markovic and Mikulic, 2010; Ache-Dias et al., 2016) enhancing the performance during

SJ and CMJ. It is well documented that plyometric training improves the mechanical power output and height during vertical jumping (Brown et al., 1986; Adams et al., 1992; Toumi et al., 2001) and, therefore, the specific gymnastic training can explain the better jumping performance of our athletes.

CONCLUSION

In conclusion, the present study demonstrated that artistic gymnastic training during preadolescence is associated with increased plantar flexor strength normalized to body mass and jumping performance, without significant effects on Achilles tendon stiffness and absolute muscle strength compared to non-athletes. The delayed maturation of the athletes and the specific training load of artistic gymnastics are likely to explain these findings. In some individual athletes, an increased level of tendon strain during maximum isometric contractions indicates imbalances between muscle strength and tendon stiffness, resulting in a high mechanical demand for the tendon. The potential contribution of the increased mechanical demand to the high risk of tendon overuse in the athletic population call for the implementation of specific exercises into the athletic training of preadolescent athletes that effectively increase tendon stiffness and support a balanced adaptation within the muscle-tendon unit. To increase tendon stiffness, we recommend exercises that combine high intensity, repetitive loading and a long duration of loading (of ≈3 s) in every repetition (for review and exemplary exercises see Mersmann et al., 2017b). Finally, with regard to the inherent limitations of cross-sectional studies, which cannot rule out genetic influences biasing the inferences made regarding the effect of training, future research should aim to investigate the effects of athletic training during preadolescence using a longitudinal design.

ETHICS STATEMENT

The study has been approved by the university ethics committee of Democritus University of Thrace, and all participants (including legal guardians) signed informed consent to the experimental procedures in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

NP and AA conceived the experiments. NP and EG performed the experiments. NP analyzed the data. FM, SB, and AA substantially contributed to data analysis. NP, FM, and AA interpreted the data. NP and AA drafted the manuscript. FM, SB, EG, and NA made important intellectual contributions during revision. All authors approved the final version of the manuscript and agreed to be accountable for the content of the work.

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Effects of Lengthening Velocity During Eccentric Training on Vastus Lateralis Muscle Hypertrophy

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Eccentric loading is an effective stimulus for muscle hypertrophy and strength gains, however, the effect of lengthening velocity is under debate. The purpose of the current study was to investigate the influence of muscle lengthening velocity during eccentric training on muscle hypertrophy and strength gains at a given overall loading volume. Forty-seven participants were randomly assigned to a control (n = 14, age: 26.9 ± 4.1 years) and an experimental group (n = 33, age: 27.1 ± 4.4 years). Each leg of the participants in the experimental group was randomly assigned to one of the four eccentric training protocols with different angular velocities (i.e., 45, 120, 210, and 300°/s). Both the magnitude of loading (100% of the isometric maximum) and overall time under tension was matched between the protocols. The training was performed for 33 sessions, 3 times per week with 5 training sets per session. Before and after the intervention, the maximum isometric knee extension moments were measured in all groups using dynamometry, vastus lateralis (VL) muscle anatomical cross-sectional area, and VL muscle volume were measured in the experimental group using magnetic resonance imaging. Data was analyzed in a mixed-design analysis of variance. After the training intervention, the maximum knee joint moments increased in the experimental group (14.2%, $p < 0.05$) but not the control group. VL anatomical cross-sectional area and VL muscle volume increased significantly ($p < 0.05$) in the experimental group (5.1 and 5.7%, respectively), but we did not find any significant differences between the four training protocols in all investigated parameters ($p > 0.05$). The present study provides evidence that muscle hypertrophy and strength gains after eccentric exercise is velocity-independent when load magnitude and overall time under tension are matched between conditions. This is likely due to the similar mechanical demand for the muscle induced by the loading conditions of all four training protocols. The better control of motion and the potentially decreased joint loading compared to high lengthening velocity contractions support the application of slow eccentric exercises in special populations like elderly and people with neurological and musculoskeletal diseases.

Keywords: muscle volume, muscle strength, eccentric training, MRI, quadriceps femoris

INTRODUCTION

Muscle strength is an important contributor to movement performance and quality of live in young, old, and diseased humans (Narici et al., 2003; Moreno Catalá et al., 2013; Mike et al., 2017). Therefore, the maintenance or increase of muscle strength is a basic target in interventions aiming to improve performance during activities of everyday living and particularly, in sports. Improvements in muscle strength are often accompanied by an increase in muscle size, i.e., muscle hypertrophy (Maughan et al., 1983; Kanehisa et al., 1994; O'Brien et al., 2009), because maximum muscle strength is directly related to the physiological cross-sectional area of the muscle (Haxton, 1944). Several training interventions with isometric (Alegre et al., 2014; Noorkõiv et al., 2015), concentric (Blazevich et al., 2007; Moore et al., 2012), eccentric contractions (Paddon-Jones et al., 2001; Shepstone et al., 2005; Moore et al., 2012), or a combination of the three contraction forms (Alegre et al., 2006; Matta et al., 2015) were able to elicit changes in both muscle strength and size (Higbie et al., 1996; Farthing and Chilibeck, 2003; Vikne et al., 2006; Blazevich et al., 2007). When comparing different forms of muscle contractions, eccentric loading was often (Seger et al., 1998; Farthing and Chilibeck, 2003; Vikne et al., 2006; Maeo et al., 2018), but not always (Higbie et al., 1996; Blazevich et al., 2007; Reeves et al., 2009; Cadore et al., 2014), found to be superior in strength improvement and increase in size when compared to training with concentric or isometric muscle loading. This superior effect is often attributed to the higher force that can be generated during eccentric contractions (Westing et al., 1988; Adams et al., 2004) due to the force-velocity relationship (Katz, 1939), resulting in a stronger loading stimulus compared to the other two contraction types. It has been reported that the disruption of muscle sarcomeres (i.e., z-line streaming) is more pronounced after bouts of eccentric contractions when compared to concentric ones (Gibala et al., 1995; Moore et al., 2005). The resultant greater muscle protein syntheses (Moore et al., 2005) and higher satellite cell activation (Crameri et al., 2004) are regarded the most likely mechanisms explaining the greater muscle hypertrophy during eccentric exercise (Franchi et al., 2017).

Earlier studies found that eccentric muscle loading with high lengthening velocity causes more severe muscle damage compared to eccentric contractions with low lengthening velocity (Shepstone et al., 2005; Chapman et al., 2006, 2008). The effect of muscle lengthening velocity after eccentric training on muscle hypertrophy and strength gains was investigated in some studies using maximum voluntary contractions (Paddon-Jones et al., 2001; Farthing and Chilibeck, 2003; Shepstone et al., 2005). However, all these studies used equal number of repetitions between the training conditions leading to a lower time under tension in the training protocol with the fast lengthening contractions. Time under tension is an important component of muscle loading and significantly affects muscle protein synthesis at given load magnitudes (Burd et al., 2012). Therefore, matching the number of repetitions between training protocols, when investigating the effect of lengthening velocity on muscle hypertrophy, may introduce limitations for the interpretation of the outcomes (Chapman et al., 2006). The previous studies (Farthing and Chilibeck, 2003; Shepstone et al., 2005) reported a trend toward greater muscle hypertrophy after eccentric training using fast lengthening contractions, despite a lower time under tension in the fast compared to slow eccentric training. Considering that the time under tension affects muscle protein synthesis (Burd et al., 2012), it can be argued that the non-significant results on the effect of lengthening velocity on muscle hypertrophy (Farthing and Chilibeck, 2003; Shepstone et al., 2005) might have been due to the differences in loading volume between the exercise protocols.

The purpose of the current study was to investigate the influence of muscle lengthening velocity during eccentric exercise on muscle hypertrophy and strength gains by matching the loading magnitude and time under tension in four exercise protocols with different lengthening velocities. We hypothesized a lengthening velocity-dependent muscle hypertrophy and strength improvement after the eccentric training period, with higher gains at faster lengthening contractions.

MATERIALS AND METHODS

Participants

Prior to the intervention, a power analysis according to a similar intervention (Sharifnezhad et al., 2014) indicated that six participants per group were required for a power of 0.95 with an effect size of 0.47 to distinguish group effects. In addition, the dropout rate in the previous intervention was about 40%; hence, we recruited 47 young active (7.4 ± 4 h of sports per week) men to ensure sufficiently sized samples. The participants were acquired from the university population by printed and digital advertising postings, organized primarily by the main experimenter (RM). We were open to include healthy adult males younger than 40 years without any musculoskeletal impairments. The participants were randomly divided into a control (n = 14, age: 26.9 ± 4.1 years, body height: 179.0 ± 5.3 cm, body mass: 74.9 ± 7.8 kg) and an experimental group (n = 33, age: 27.1 ± 4.4 years, body height: 179.6 ± 6.4 cm, body mass: 73.7 ± 8.8 kg). All participants were informed about the aim and the methods of the study and gave their written informed consent to participate in the investigation. The study was performed in accordance with the declaration of Helsinki and approved by the ethics board of the Humboldt-Universität zu Berlin (EA2/076/15).

Experimental Design

The participants of the experimental group performed eccentric isokinetic contractions of the knee extensors in a dynamometer (Biodex System 3, Biodex Medical, Inc., Shirley, NY, USA) for 33 trainings sessions, with 3 sessions per week on separate days and 5 training sets per session. The control group did not receive any specific training. The left and right legs of the participants in the experimental group were randomly allocated to one of four training protocols that differed in the movement velocity (protocol p45, angular velocity 45°/s, n = 16; p120,

angular velocity 120°/s, n = 16; p210, angular velocity 210°/s, n = 17; p300, angular velocity 300°/s, n = 17). The loading magnitude (100% of the isometric maximum voluntary contraction, iMVC) and range of motion (25–100° of knee joint angle; 0° = full extension) were constant between protocols. After each repetition, the leg was returned to the starting position by the participant in the same angular velocity as during loading. Depending on the training velocity, the return of the lever took 1.66 s (p45), 0.63 s (p120), 0.36 s (p210), and 0.25 s (p300), respectively. The training protocols were designed to provide an equal loading volume (i.e. integral of the knee joint moment over the time), and therefore, the number of repetitions was adjusted accordingly, resulting in 3, 8, 14, and 20 repetitions for the protocols p45, p120, p210, and p300, respectively, which provides a similar overall time under tension in all protocols. A 2–3 min break was allowed between the training sets. Training with eccentric contractions is likely to induce muscle soreness (Proske and Morgan, 2001; Chapman et al., 2008). Therefore, we started the training with a rather low loading magnitude of 65% iMVC and gradually increased the load to finally 100% iMVC within the first two training weeks in all experimental protocols. During each training session, the participants were provided with a visual feedback of the target and their generated moment during the eccentric knee extension exercise, to generate the appropriate load magnitude. Due to the higher force potential of a muscle during an eccentric contraction compared to isometric ones, the participants were able to complete all repetitions according to the protocol requirements (i.e., 100% iMVC). The angle-specific target moment was calculated based on a second order polynomial representing the moment-angle relationship. The polynomial was determined in relation to the individual iMVC measurements at 30, 65, and 100° of the knee joint angle. The iMVC measurements were repeated every sixth training session to ensure a progression in the load magnitude.

The pre and post measurements were done in two separate sessions, 1–3 days apart. In the first session, the vastus lateralis (VL) muscle morphology was measured using magnetic resonance imaging (MRI). During the second session, the maximum knee joint moment of the knee extensors was measured. The MRI-data were collected prior to the measurement of the maximum isometric moment to avoid any bias on the assessment of muscle morphology, e.g., due to muscle swelling from exercise (Clarkson and Hubal, 2002). Further, the post measurements were performed 7–10 days after the last training session, to exclude fatigue effects from the training (Chapman et al., 2006). For cost and time efficiency, the MRI measurements were only performed with the experimental group. Previous studies reported no size-alterations of the quadriceps muscles when training conditions were unchanged (Higbie et al., 1996). A timeline of the measurement is provided in **Figure 1**.

Muscle Morphology Measurement

The VL muscle was measured and reconstructed using an open 0.25 Tesla MRI scanner (G-Scan, Esaote, Genua, Italy) with the procedure described in detail by Marzilger et al. (2019). Briefly, since the scanner had a small field of view of approximately 20 cm × 20 cm × 20 cm, the thigh was separated with markers (elastic straps with three fish oil capsules) into three sections (distal, medial, and proximal part; **Figure 2**) based on the femur length and each of these sections was measured separately. The participants were positioned supine in the scanner and three separate Turbo 3D T1 weighted sequences (TE: 16 ms, TR: 39 ms, slice thickness: 3.1 mm, no gaps) from the thigh were acquired. Between the single sequences, the participants were manually repositioned to locate the muscle section of interest into the imaging region of the scanner. To avoid any dislocation of the markers during the repositioning process, the markers were secured with tape and the position was highlighted with a skin marking pen. The segmentation of the MRI images was performed offline using Osirix (version 4.0, Pixmeo, Geneva, Swiss). In a first step, the border (epimysium) of VL was manually segmented in every muscle section (i.e., distal, medial, and proximal). In the second step, the three markers were manually identified in each muscle sequence and an overlap of at least three slices between two sequences (e.g., proximal and medial) was generated. Finally, a custom-written Matlab (2012, The Mathworks, Natick, USA) algorithm calculated the overlap of two different muscle sections due to the position of the identified markers and reconstructed the entire muscle volume from all three muscle sections (Marzilger et al., 2019). From the reconstructed VL muscle, we examined muscle volume, maximum anatomical cross-sectional area ($ACSA_{Max}$) and muscle ACSA in 10% intervals along the muscle length. Due to the small field of view of the MRI scanner, we measured the volume of the VL as representative for the knee extensors due to its great volume and the resultant small relative measurement error in the segmentation process.

FIGURE 1 | Timeline of the experimental protocol. MRI, magnetic resonance imaging; VL, vastus lateralis; iMVC, isometric maximum voluntary contraction.

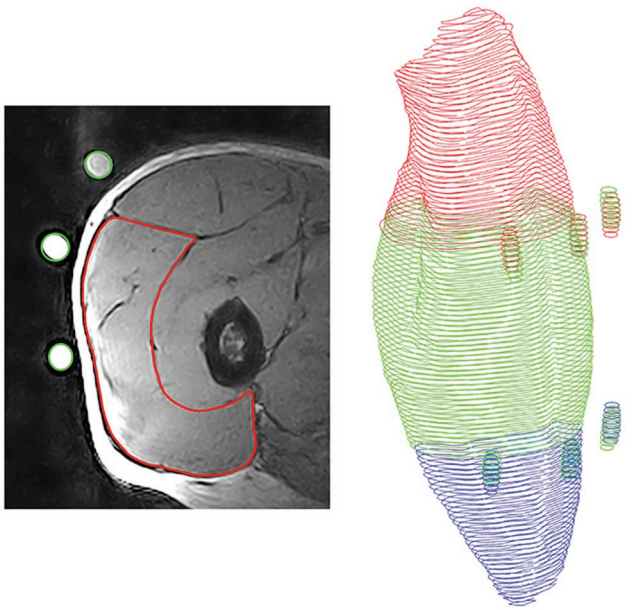

FIGURE 2 | Procedure of muscle reconstruction. Left: Magnetic resonance image of the vastus lateralis with segmented muscle (red) and identified fish oil capsules (green); Right: Reconstructed muscle with fish oil capsules, proximal part (red), medial part (green) and distal part (blue).

Maximum Knee Joint Moment Measurement

After 10 min of standardized warm-up, including 5 min cycling on an ergometer, as well as several squats and countermovement jumps the participants were seated in the same dynamometer as for the training. The hip angle was set to 85° to reduce the contribution of the bi-articular rectus femoris to the resultant knee joint moment (Herzog et al., 1990). In the following, the participants performed several (in average 4–5) isometric contractions as specific warm-up. The muscle strength was measured during single repetitions of maximal isometric voluntary contractions in five different knee joint angles (60, 65, 70, 75, and 80°). We used these knee joint angles because in this range of motion, the knee extensor muscles generate the maximum joint moment (Mersmann et al., 2014; Sharifnezhad et al., 2014). Due to soft tissue deformation and dynamometer compliance, the real knee joint angles at the plateau of the achieved maximum knee joint moment are different (Arampatzis et al., 2004). Therefore, the measured moments of the dynamometer were corrected for gravitational forces and axis misalignment using the inverse dynamic approach suggested by Arampatzis et al. (2004). For these calculations, the kinematics of each leg was captured with seven cameras (six MX-20 and one F-20) of a vicon motion capture system (Nexus 1.6, Vicon, Stadt, UK). The markers were fixed at the following anatomical landmarks: medial and lateral malleolus, medial and lateral femur condyle, trochanter major and spinae iliaca anterior. Finally, the resultant moments were normalized to body mass and fitted with the corresponding knee joint angles using a second order polynomial to determine the maximal knee joint moment.

Statistics

All investigated parameters were tested for normal distribution using the Kolmogorov-Smirnov test. A mixed-design analysis of variance for repeated measures (split-plot ANOVA) was performed with time as within-subjects variable (pre vs. post) and exercise protocols as between-subjects factor (p45, p120, p210, p300, and control group). A Bonferroni-Holm corrected *post-hoc* analysis was conducted in the case of a significant interaction of the factors time and protocol. To check the anthropometrical, functional, and morphological parameters of the included participants in each group as well as the percentage pre-to-post changes of muscle morphology and knee joint moment, we used a one-way ANOVA and Bonferroni *post-hoc* comparisons between the groups. The level of significance for all statistical tests was set to $\alpha = 0.05$. In case of significant main effects and interactions, we calculated the partial eta square (η^2). For respective *post-hoc* observations, we determined Cohen's *d* effect size for the difference between pre and post values for single protocols.

RESULTS

Thirteen control participants and 28 participants of the experimental group successfully finished the training and underwent the post measurement, yielding to $n = 14$ participants for each investigated protocol. There was no main effect of group in age ($p = 0.562$), height ($p = 0.872$), and body mass ($p = 0.860$) at the beginning of the intervention. Further, we did not observe any statistically significant changes in body height ($p = 0.762$) and body mass ($p = 0.157$) following the training period.

The baseline values for all measured parameters are presented in **Table 1**. Before the training intervention, there were no between-group differences in the maximum knee joint moment ($p = 0.733$), $ACSA_{Max}$ ($p = 0.570$), and muscle volume ($p = 0.692$). The achieved maximum isometric knee extension moment demonstrated a significant effect of time ($p < 0.001$, $\eta^2 = 0.55$), with a significant time-by-protocol interaction ($\eta^2 = 0.19$, **Table 1**). The *post-hoc* comparisons showed significant changes in maximum knee joint moment after the training in all experimental protocols but not in the control group (**Table 1, Figure 3**). The relative changes of maximum moment in the experimental groups (in average 14.2 ± 11.1%) were statistically significant ($p < 0.05$, *post-hoc* comparisons) greater compared to the control group but without any differences ($p > 0.05$) between the training protocols (**Figure 3**). The ANOVA for both morphological parameters (i.e., $ACSA_{Max}$ and muscle volume) showed a significant effect of time ($p < 0.001$, $\eta^2 = 0.55$ for $ACSA_{Max}$ and $\eta^2 = 0.65$ for muscle volume), but no time-by-protocol interaction, indicating no differences in the training-induced alterations between the four training protocols. The average increases in $ACSA_{Max}$ and muscle volume were 5.1 ± 4.7% and 5.7 ± 4.6%, respectively (**Figure 4**). In a similar manner,

TABLE 1 | Mean ± SD of the maximum knee joint moment ($Moment_{Max}$), maximum anatomical cross-sectional area ($ACSA_{Max}$) and muscle volume for the control (CG) and experimental groups (p45, protocol with 45°/s; p120, protocol with 120°/s; p210, protocol with 210°/s; p300, protocol with 300°/s); *d*, Cohen's *d*; Range, range of change.

		Control group	Experimental group			
		CG	p45	p120	p210	p300
$Moment_{Max}$ (Nm/kg)†,#	Pre	3.7 ± 0.5	3.7 ± 0.4	3.9 ± 0.4	3.9 ± 0.4	3.9 ± 0.4
	Post	3.7 ± 0.4	4.3 ± 0.5*	4.4 ± 0.4*	4.4 ± 0.7*	4.4 ± 0.5*
	Range	–0.3–0.3	–0.2–1.1	–0.1–1.1	–0.4–2.0	–0.2–1.1
	d		1.213	1.293	0.921	1.072
$ACSA_{Max}$ (cm^2)†	Pre	–	35.9 ± 2.7	34.4 ± 4.9	34.4 ± 5.4	33.5 ± 4.8
	Post	–	37.5 ± 3.2	36.1 ± 4.8	36.4 ± 6.0	35.3 ± 5.5
	Range		–1.3–4.4	–2.3–5.5	0.1–3.7	–1.3–5.6
	d		0.505	0.347	0.273	0.320
Muscle volume (cm^3)†	Pre	–	744.0 ± 82.5	715.4 ± 113.6	742.4 ± 143.9	699.1 ± 118.1
	Post	–	780.9 ± 89.6	752.9 ± 108.1	784.8 ± 143.7	739.9 ± 121.0
	Range		–6.4–81.3	–20.2–112.5	–4.8–71.8	–43.7–85.9
	d		0.416	0.334	0.297	0.342

†*Statistically significant main effect of time ($p < 0.05$).*

#*Statistically significant time-by-protocol interaction ($p < 0.05$).*

**Statistically significant differences (post-hoc analysis) between pre und post measurement ($p < 0.05$).*

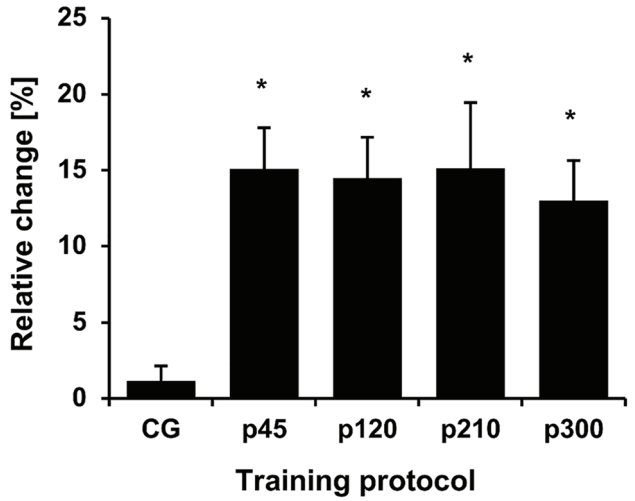

FIGURE 3 | Relative change of the maximum knee joint moment normalized to body mass. The relative changes with regard to baseline are shown on the *y*-axis, while the different protocols are given on the *x*-axis. CG, control group; p45, protocol with 45°/s; p120, protocol with 120°/s; p210, protocol with 210°/s; and p300, protocol with 300°/s. *Statistically significant differences to control group ($p < 0.05$).

there was a significant increase ($p < 0.001$ to $p = 0.008$) of the ACSA in every 10% interval (except from 0 to 10%) along the VL muscle length without any time-by-protocol interactions ($p > 0.05$; **Figure 5**).

DISCUSSION

The present study shows that an exercise intervention using eccentric contractions with a high load magnitude (100% of iMVC) and a relative large range of motion (75°) provides an effective stimulus for VL muscle hypertrophy of up to 5% in 11 training weeks and can improve knee extensors muscle strength by up to 14%. Since no differences between the training protocols were observed, the results are in contrast to our hypothesis and provide evidence that muscle hypertrophy is independent of the contraction velocity applied during eccentric exercise. Therefore, our hypothesis of a lengthening velocity-dependent muscle hypertrophy needs to be rejected.

Several studies found an increase in maximum muscle strength (Farthing and Chilibeck, 2003; Vikne et al., 2006; Blazevich et al., 2007; Moore et al., 2012), anatomical cross-sectional area (Higbie et al., 1996; Vikne et al., 2006; Moore et al., 2012), and muscle volume (Blazevich et al., 2007) after training with eccentric contractions. Some earlier studies investigating the effects of lengthening velocity on muscle adaptation after a period of training concluded that eccentric exercise using fast lengthening contractions (i.e., 180 or 210°/s) lead to greater muscle hypertrophy and strength gains compared to slow (i.e., 20 or 30°/s) contractions (Paddon-Jones et al., 2001; Farthing and Chilibeck, 2003; Shepstone et al., 2005). The greater muscle damage that usually occurs during the fast lengthening contractions and the resultant greater muscle remodeling and greater duration of protein synthesis (Farthing and Chilibeck, 2003; Shepstone et al., 2005) was suggested as a possible mechanism for this phenomenon (i.e., fast eccentric contractions being more effective for muscle hypertrophy and strength gains). A greater muscle hypertrophy during the fast lengthening contractions (Farthing and Chilibeck, 2003; Shepstone et al., 2005) was, however, not statistically confirmed (both studies found only a trend toward greater muscle hypertrophy). Furthermore, in these earlier studies, the time under tension during the training was different between protocols, and therefore, it is not possible to isolate the effects of the lengthening velocity on muscle hypertrophy. Unequal times under tension in muscle loading result in a different stimulation of muscle

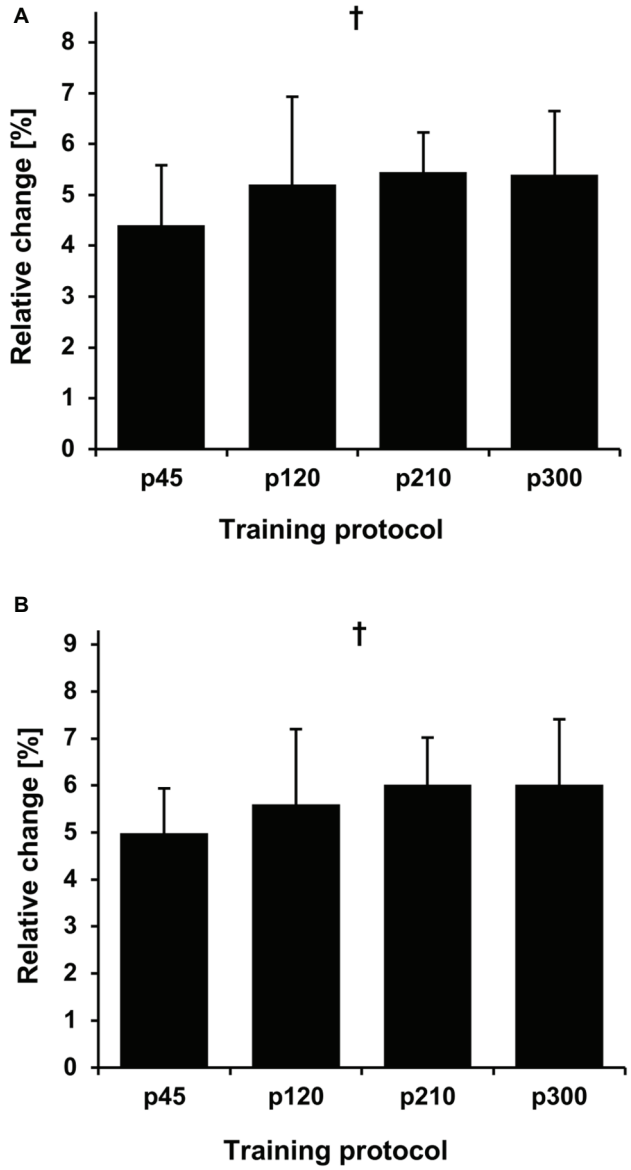

FIGURE 4 | Relative change in maximum anatomical cross-sectional area ($ACSA_{Max}$) **(A)** and muscle volume **(B)** in the four training protocols. The relative changes with regard to baseline are shown on the *y*-axis, while the different protocols are given on the *x*-axis. The error bars indicate the standard error. p45, protocol with 45°/s; p120, protocol with 120°/s; p210, protocol with 210°/s; and p300, protocol with 300°/s. †Statistically significant main effect of time ($p < 0.001$).

protein synthesis, which in turn affects muscle hypertrophy after exercise (Burd et al., 2012).

In our experiment, we applied the same level of load magnitude and total time under tension on the knee extensor muscles and we used an equal range of motion during the eccentric contractions. The only difference between the four investigated exercise protocols was the lengthening velocity. The angular velocities ranged from 45 to 300°/s and represent a wide range of lengthening velocities. As there is a good reason to assume that training volume shows a direct influence on training outcomes (Mitchell et al., 2012), we can argue that the used experimental design is most suitable to investigate the isolated effects of lengthening velocity on muscle hypertrophy and muscle strength gains after eccentric training and, to our knowledge, this is the first longitudinal study applying this approach. Additional to the similar increase in muscle volume and muscle strength in all four protocols, we did not find any specific regional muscle hypertrophy in any of the different protocols as reported in other studies investigating hypertrophic muscle responses by comparing eccentric and concentric training (Blazevich et al., 2007; Franchi et al., 2014). Although the absolute ACSA in every 10% interval along the muscle length increased, we did not find any differences between the protocols.

Our findings provide evidence for an independence of the exercise-induced muscle hypertrophy and muscle strength from lengthening velocity and support the idea that magnitude of loading (Sharifnezhad et al., 2014), muscle length at which the load is applied (Sharifnezhad et al., 2014; Noorkõiv et al., 2015) and time under tension might be the main determinants of muscle adaptation after eccentric training. We hypothesized that muscle hypertrophy would be preferentially increased in the training protocols with the high lengthening velocities based on the reports of greater muscle damage in fast eccentric contractions (Shepstone et al., 2005; Chapman et al., 2006) and the resultant greater muscle remodeling (Farthing and Chilibeck, 2003; Shepstone et al., 2005). However, the damage-based muscle hypertrophy is currently in debate and there is experimental evidence of muscle hypertrophy without previous muscle damage (Schoenfeld, 2012; Hyldahl and Hubal, 2014; Franchi et al., 2017). It has been reported that the titin kinase domain can be directly activated by mechanical force to trigger signaling independent of muscle damage after eccentric loading (Lange et al., 2006; Puchner et al., 2008). Furthermore, it is well known that muscles adapt to the exercise-induced damage after eccentric loading, the so called repeated bout effect, which provides protection against further muscle damage (Nosaka and Clarkson, 1995; Hyldahl et al., 2015). Neural adaptations in the motor unit recruitment, extracellular matrix alterations initiated by the activation of specific extracellular matrix signaling pathways, changes in the fascicle kinetics and increased inflammation sensitivity are important mediators to the protective effects against further muscle damage (Hyldahl et al., 2015, 2017). In our study, the participants successfully completed 33 training units over 11 weeks of eccentric training at 100% of the iMVC. Although we did not investigate muscle damage in our experiment, we can assume that, due to the repeated bout effect and the protective muscle adaptation against eccentric loading-induced damage the observed muscle hypertrophy was not mainly due to muscle damage. Recent studies (Franchi et al., 2014, 2015) reported similar muscle hypertrophy gains and similar long-term muscle protein synthesis after eccentric and concentric training when the relative load was matched between the two different contraction modes. We argue that the matched loading magnitude, time under tension, and range of motion used in our protocols were the reasons for the similar and velocity-independent hypertrophic responses in the current investigation.

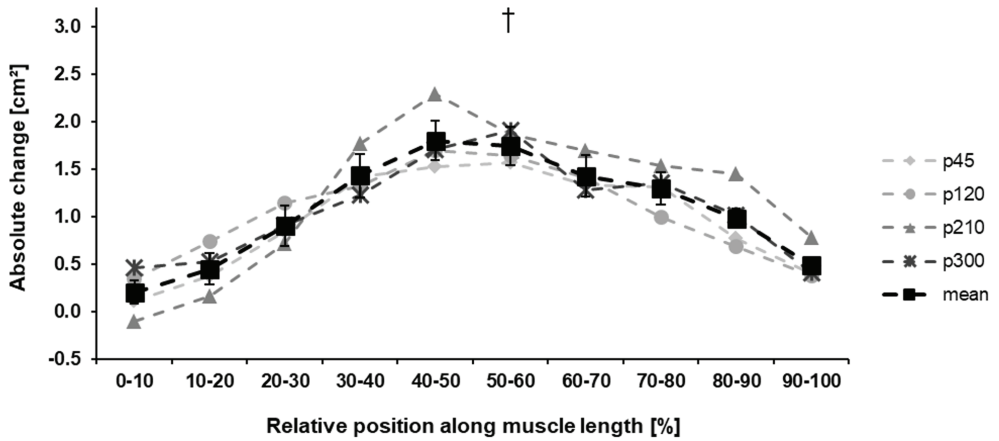

FIGURE 5 | Absolute change ±SE of the anatomical cross-sectional area (ACSA) of the vastus lateralis in the four training protocols along the muscle length. The solid black line (mean) indicates the average change in muscle ACSA of all training protocols (p45, protocol with 45°/s; p120, protocol with 120°/s; p210, protocol with 210°/s; and p300, protocol with 300°/s.). To improve the visibility, the standard error is only shown in the mean data curve. †Statistically significant main effect of time ($p < 0.001$).

The increase in muscle strength can be attributed to both the observed muscle hypertrophy and neural adaptations (Higbie et al., 1996; Paddon-Jones et al., 2001; Douglas et al., 2017). The greater effects observed on strength compared to the morphological changes suggest a considerable neural contribution. However, as both muscle hypertrophy and strength development were similar between protocols, we also would expect a similar contribution of neural adaptation, which is likely explained by the same intensity (in terms of force production) across protocols (Campos et al., 2002). Though in our study no differences in the hypertrophic response between protocols were observed, it might still be that structural changes within the muscle occurred dependent on the lengthening velocity during exercise. It has been reported that after acute eccentric loading mostly the fast glycolytic fiber type demonstrated histologic abnormalities (Fridén et al., 1983; Lieber and Fridén, 1988), resulting in selective increased satellite cell activation (Cermak et al., 2013; Hyldahl et al., 2014) and hypertrophy of type II fibers (Hortobágyi et al., 2000; Verdijk et al., 2009). The selective fiber type II specific hypertrophy after long term eccentric exercise is pronounced at fast lengthening contractions (Paddon-Jones et al., 2001; Shepstone et al., 2005). However, to what extent fiber type-specific hypertrophy contributed to the overall similar increases in muscle volume of the four training protocols cannot be answered with our present study design.

Due to the present study design, a possible cross-education effect between limbs cannot be ruled out completely. However, there is no scientific evidence for interlimb transfer effects in terms of exercise-induced muscle hypertrophy (Lee and Carroll, 2007; Hendy and Lamon, 2017). Thus, any possible transfer effect is limited to strength gains as a result of neural adaptation. Yet, neural adaptation shows considerable specificity to the type and velocity of a muscle contraction (Hortobágyi et al., 1996; Dos Santos Rocha et al., 2011), which suggests a reduced contribution of cross-education as a result of eccentric training on isometric strength as measured in our study. Moreover, cross-education shows strong effects on untrained contra-lateral limbs, yet if the contra-lateral limb also receives training, it seems likely that the neural activity associated to it largely eliminates the cross-education effect. Moreover, the combination of the exercise protocols within the current intervention was completely randomized between participants, and thus, any combination of protocols for the two legs was possible (e.g., 45 and 300°/s or 45 and 210°/s or 210 and 120°/s, …). Therefore, any possible cross-transfer effects would also be randomly distributed over the applied protocols and not systematically bias our results.

In conclusion, the current study demonstrated that muscle hypertrophy after eccentric training interventions is velocity-independent when time under tension is matched between conditions and the load magnitude and range of motion are similar. Gains in muscle volume directly affect the maximum muscle power (Narici, 1999; O'Brien et al., 2009), which is of major importance for both sports performance in disciplines involving jumping and sprinting (Cronin and Sleivert, 2005) as well as for mobility functions in the elderly (Tschopp et al., 2011; Izquierdo and Cadore, 2014). However, lower movement velocities allow for a better control of motion and lower fluctuations of accelerations during eccentric contractions (Christou et al., 2003), which might reduce peak forces in the involved joints given that controlled movement behavior plays a major role for joint loads (Bergmann et al., 2004). Further, the longer time under tension during single repetitions in slow eccentric training might provide a superior stimulus for tendon adaptation compared to fast eccentric movements (Arampatzis et al., 2010; Bohm et al., 2014), which might be important for the prevention of imbalances of muscle and tendon adaptation (Mersmann et al., 2017, for a review). Therefore, we argue that the application of eccentric exercises with low angular velocities could be more applicable for muscle training in populations like elderly, people with neurological and musculoskeletal diseases or athletes at risk for tendinopathy (Hyldahl and Hubal, 2014; Mersmann et al., 2017).

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

All participants were informed about the aim and the methods of the study and gave their written informed consent to participate in the investigation. The study was performed in accordance with the declaration of Helsinki and approved by the ethics board of the Humboldt-Universität zu Berlin (EA2/076/15).

AUTHOR CONTRIBUTIONS

RM and AA conceived the experiment. RM and SB performed the experiments. RM analyzed the data and SB, FM, and AA substantially contributed to data analysis. RM, SB, and AA interpreted the data. RM and AA drafted the manuscript, and SB and FM made important intellectual contributions during revision. All authors approved the final version of the manuscript and agree to be accountable for the content of the work.

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frontiers in Physiology

ORIGINAL RESEARCH
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Patellar Tendon Strain Associates to Tendon Structural Abnormalities in Adolescent Athletes

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High mechanical strain is thought to be one of the main factors for the risk of tendon injury, as it determines the mechanical demand placed upon the tendon by the working muscle. The present study investigates the association of tendon mechanical properties including force, stress and strain, and measures of tendon micromorphology and neovascularization, which are thought to be indicative of tendinopathy in an adolescent high-risk group for overuse injury. In 16 adolescent elite basketball athletes (14–15 years of age) we determined the mechanical properties of the patellar tendon by combining inverse dynamics with magnetic resonance and ultrasound imaging. Tendon micromorphology was determined based on a spatial frequency analysis of sagittal plane ultrasound images and neovascularization was quantified as color Doppler area. There was a significant inverse relationship between tendon strain and peak spatial frequency (PSF) in the proximal tendon region ($r = -0.652$, $p = 0.006$), indicating locally disorganized collagen fascicles in tendons that are subjected to high strain. No such associations were present at the distal tendon site and no significant correlations were observed between tendon force or stress and tendon PSF as well as between tendon loading and vascularity. Our results suggest that high levels of tendon strain might associate to a micromorphological deterioration of the collagenous network in the proximal patellar tendon, which is also the most frequent site affected by tendinopathy. Neovascularization of the tendon on the other hand seems not to be directly related to the magnitude of tendon loading and might be a physiological response to a high frequency of training in this group. Those findings have important implications for our understanding of the etiology of tendinopathy and for the development of diagnostical tools for the assessment of injury risk.

Keywords: tendon injury, overload, micromorphology, imbalance, youth athletes

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INTRODUCTION

The human muscle-tendon unit is highly adaptive to its mechanical environment. In response to increased mechanical loading, the strength capacity of a muscle may change due to an increased activation level, hypertrophy and/or specific tension (Narici et al., 1989; Aagaard et al., 2002; Erskine et al., 2010). Tendons transmit the forces generated by the muscle to the skeleton and function as

biological springs. Due to their elasticity, tendons are able to store and release mechanical strain energy and provide favorable operating conditions for the muscle considering the force-length-velocity relationship of the muscle fibers (Kawakami and Fukunaga, 2006; Lichtwark and Wilson, 2007; Bohm et al., 2018). They are also able to adapt to loading by increasing their stiffness based on changes of their material properties and/or (in the long-term) radial growth (Bohm et al., 2015, for review). A balanced development of muscle strength and tendon stiffness is not only necessary to maintain the operating conditions of muscle fascicles in an optimal range, but also prevent damage to the tendinous tissue that is now subject to higher muscular forces, as the maximum tolerable strain of tendons is quite constant (LaCroix et al., 2013; Shepherd and Screen, 2013). An imbalanced adaptation of muscle and tendon could, therefore, pose a risk of overload injury (Mersmann et al., 2017a). However, muscle and tendon might not necessarily adapt in a balanced manner. The rate of tissue renewal is markedly lower in tendons (Heinemeier et al., 2013b), which might cause a delayed adaptive response with regard to the muscle (Kubo et al., 2010, 2012). Further, the mechanical stimuli that efficiently promote tissue adaptation show differences between muscle and tendon (Arampatzis et al., 2007a; Heinemeier et al., 2013a). During growth, processes of maturation could additionally challenge the development within the muscle-tendon unit (Neugebauer and Hawkins, 2012; Mersmann et al., 2017a). In fact, recent investigations on adolescent volleyball athletes, which is a high-risk group for the development of tendinopathy, provided evidence that an imbalance in the development of muscle and tendon results in an increase of *in vivo* strain of the patellar tendon during maximum voluntary muscle contractions (Mersmann et al., 2014, 2016, 2017b). An increase of tendon strain during maximum effort muscle activity implies an increased mechanical demand for the tendon. It has been shown in cadaveric experiments on human Achilles tendons that time until failure in cyclic loading directly depends on the initial strain induced by the applied load (Wren et al., 2003). Further, there is now growing evidence available that, at least in the Achilles tendon, patients with tendinopathy demonstrate higher levels of strain during contractions (Obst et al., 2018). However, the implications of the increased mechanical demand for the patellar tendon, as observed in adolescent athletes, for the risk of tendon injury so far remain an assumption.

Tendinopathy is frequently associated with tendinosis, which describes degenerative processes and abnormalities within the tendon matrix, including disorganization of the collagenous network, vascular infiltration, increased cellularity and ground substance (Khan et al., 1996; Abat et al., 2017). Some of the consequences of these changes can be evaluated using ultrasound imaging. The assessment of tendon thickening, hypoechogenicity, and vascularity are now established tools for confirming the diagnosis of tendinosis in the clinical setting (Campbell and Grainger, 2001; Warden et al., 2006) and show high reliability (Black et al., 2004; Cook et al., 2005). It even seems that structural abnormalities in asymptomatic tendons can predict the development of future symptoms (in terms of occurrence, not severity), with athletes that show indications for tissue degeneration in the ultrasound examination of the patellar tendon are about fourfold more likely to become symptomatic (McAuliffe et al., 2016). Analysis algorithms for ultrasound image post-processing further enable the quantification of the packing density and orientation of collagen bundles and provide a possibility to non-invasively estimate the structural integrity of the tissue (Bashford et al., 2008; van Schie et al., 2010). With this approach it has been demonstrated that not only is the collagenous scaffold of the tendon disorganized in patients with tendinopathy (Kulig et al., 2013), but the level of disorganization predicts to some extent the mechanical properties of tendons (Kulig et al., 2016). Therefore, it seems well-possible that in individuals with a high risk for tendinopathy, an increased mechanical demand for the tendon (i.e., strain) is associated to structural precursors of the pathology. Since progressive damage to collagen fibrils and fibers reduces the intrinsic extracellular matrix tension and leads to a deterioration of the tendon mechanical properties upon strain-induced overload (Fung et al., 2009; Pingel et al., 2014), estimates of tendon micromorphology might further predict the tendon elastic modulus (Kulig et al., 2016).

Considering the increased levels of patellar tendon strain that have been observed in adolescent athletes at risk of tendinopathy and the structural changes that have been reported to predict tendinopathy, the present study aims to investigate if there is an association between the mechanical and structural characteristics of the patellar tendon that particular group. We hypothesized that while tendon force and stress would not show a direct association to structural abnormalities, the strain as a measure of tendon mechanical demand would predict the structural integrity and occurrence of neovascularization in the patellar tendon. Moreover, we expected the material properties of the patellar tendon to associate with its micromorphology.

MATERIALS AND METHODS

Participants and Experimental Design

Sixteen male adolescent elite basketball players were recruited for the present study. Inclusion criteria were an age of 14 or 15 years, regular participation in basketball training at least four times a week and no neurological or musculoskeletal impairments relevant for the purpose of the study. Mean ± standard deviation of age, body height and mass were 14.8 ± 0.5 years, 185.1 ± 8.2 cm and 72.4 ± 9.4 kg, respectively. Maturity was predicted using age and sitting height (90.1 ± 4.8 cm) in the recalibrated prediction equation for boys suggested by Moore et al. (2015), yielding an estimated average offset from the peak height velocity (PHV) of 0.9 ± 0.7 years. Most players were asymptomatic, yet we included six participants that reported patellar tendon pain during sportive and/or everyday activity, which was assessed using the VISA-P questionnaire (Visentini et al., 1998). However, none of them matched the exclusion criteria that they would not be able to exert maximum isometric voluntary contractions due to pain. One participant reported a history of Osgood–Schlatter disease, yet he experienced no symptoms at the distal attachment in the last 3 month and no

signs of structural abnormalities were observed in the ultrasound examination. The participants and legal guardians gave written informed consent to the experimental procedures, which were approved by the ethics committee of the Humboldt-Universität zu Berlin and carried out in accordance with the declaration of Helsinki. All measurements were performed on the dominant leg, which was determined by asking for which leg would be used for kicking a ball. The measurements were done on two separate days, with the magnetic resonance imaging session scheduled not more than 1 week before or after the ultrasound/dynamometry assessment. The assessment of tendon micromorphology and vascularity was performed prior to the one of tendon mechanical properties to avoid acute loading-related responses.

Assessment of Tendon Morphology and Moment Arm

To determine the cross-sectional area (CSA) and moment arm of the patellar tendon, magnetic resonance images (MRIs) of the participants were captured while lying supine with the dominant knee flexed to 10° (0° = full extension) in a 0.25 T MRI scanner (G-Scan, Esaote, Italy). The knee angle was chosen to reduce the slack of the tendon, which facilitates the subsequent tendon segmentation. In transverse MRI sequences [3D HYCE (GR), 10 ms repetition time, 5 ms excitation time, 80° flip angle, 3 mm slice thickness, one excitation] the boundaries of the patellar tendon were segmented in OsiriX (Version 7.0.2, Pixmeo SARL, Bernex, Switzerland) between the distal apex of the patellar and deep insertion at the tibial tuberosity. As recommended by Couppé et al. (2013), we used the NIH color scale during the segmentation to increase the accuracy of tracing the contours of the tendon. Since it is barely possible to perfectly align the longitudinal axis of the tendon with the longitudinal axis of the MRI scanner, simple transversal plane segmentations would lead to an overestimation of the CSA. Therefore, the digitized patellar tendon CSAs were transformed orthogonal to the line of action of the patellar tendon, which was defined as the line of best fit through the geometrical centers of the respective CSAs (**Figure 1A**).

For the assessment of the tendon moment arm, we tracked the contours of the posterior part of the femur condyles in sagittal plane sequences of a similar set 3D HYCE scan protocol. A least-squares circular fit was applied to the segmentation and the line connecting the centers of the circle representing the medial and lateral condyle respectively was the approximated rotation axis of the knee (Churchill et al., 1998). The perpendicular distance from the patellar tendon line of action to the axis of rotation represented the patellar tendon moment arm (with regard to the measurement-position of 10° knee flexion).

Assessment of Tendon Mechanical Properties

The mechanical properties of the patellar tendon were determined based on its force-elongation relationship, which was assessed for isometric contractions by combining inverse dynamics, electromyography and ultrasound imaging. For the kinematic recordings with a motion capture system (Nexus version 1.7.1; Vicon Motion Systems, Oxford, United Kingdom), integrating eight cameras (6x F20, 2x T20) operating at 250 Hz, five reflective markers were fixed to the lateral and medial malleolus, femoral epicondyles and the greater trochanter. Further, for estimating the contribution of the antagonistic muscles during the isometric contractions (Mademli et al., 2004), two bipolar surface electrodes (Blue Sensor N, Ambu GmbH, Bad Nauheim, Germany) were fixed over the mid-portion of the muscle belly of the lateral head of the biceps femoris with an inter-electrode distance of 2 cm after shaving and cleaning the skin. The electromyographic (EMG) activity of the lateral head of the biceps femoris was recorded wirelessly (Myon m320RX, Myon AG, Baar, Switzerland) and integrated into the Vicon system.

Following a standardized warm-up including 10 submaximal isometric contractions as familiarization with the dynamometer (Biodex System III, Biodex Medical, Inc., Shirley, NY, United States), the participants performed three trials of isometric maximum voluntary knee extension contractions (iMVC) at 85° trunk flexion (supine = 0°) and resting knee joint angles of 65 to 75° based on the dynamometer feedback (0° = full extension) in 5° intervals. These resting angles were chosen as, in our experience, the optimum knee angle for force production is usually reached during contraction from a rest position within this range. Subsequently, a 10 cm probe of the ultrasound system

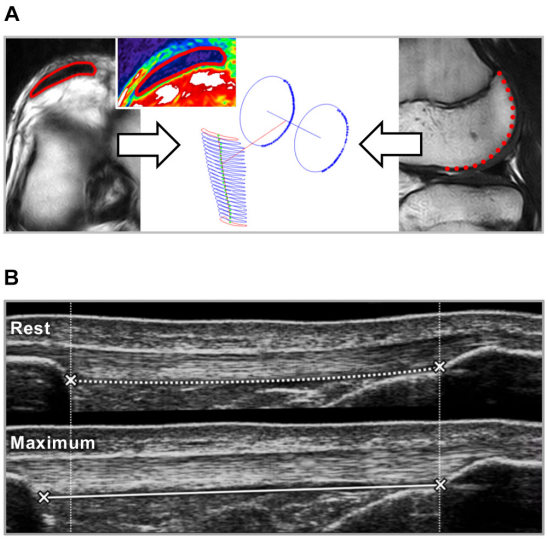

FIGURE 1 | (A) Patellar tendon cross-sectional area (CSA) and moment arm were determined based on magnetic resonance images. In transverse plane images the tendon CSA (red) was segmented over the full length of the tendon (left; inset picture shows the tendon in NIH color scale). Sagittal plane segmentations of the posterior contours of the lateral and medial femur condyles were approximated using a circular fit and the line connecting the centers of the circles was defined as rotation axis (Churchill et al., 1998). The perpendicular distance to the tendon line of action represents the tendon moment arm. **(B)** The elongation of the patellar tendon during isometric ramp contractions from rest to maximum was measured using ultrasound. Tendon strain, stiffness and elastic modulus were calculated based on the force-elongation and stress-strain relationship between 50 and 80% of the maximum tendon force or stress.

(LA923, 7.5 MHz) was fixed in alignment with the longitudinal axis of the patellar tendon with a modified knee brace. The tendon elongation was captured during five trials of isometric ramp contractions (i.e., steadily increasing effort from rest to 90% of the iMVC in about 5 s). The joint angle was the one in which the highest individual iMVC was achieved, to account for the interindividual variation in optimum angle. To be able to account for moments of gravity in the inverse dynamics approach applied to calculate joint moments (Arampatzis et al., 2004) an additionally knee extension trial (driven by the dynamometer at 5°/s) was recorded. Finally, two trials of knee flexion contractions were recorded and to establish an activation-flexion moment relationship that was used to estimate the knee flexion moments generated during isometric contractions by the antagonists when calculating the knee extension moments (Mademli et al., 2004).

To calculate patellar tendon forces, the knee extension moments were divided by the tendon moment arm, which was adjusted to the respective knee joint angle based on the data reported by Herzog and Read (1993). The maximum tendon force (TF_{max}) refers to the force calculated for the maximum iMVC trial of each participant. The elongation of the tendon during the ramp contractions was determined by tracking the displacement of the deep insertion of the tendon at the patella and tibial tuberosity using a semi-automatic software (Tracker Video Analysis and Modeling Tool V. 5.06, Open Source Physics, Aptos, CA, United States; see **Figure 1B** for a schematic illustration). The force-elongation relationship of the five trials of each participant was averaged to achieve an excellent reliability (Schulze et al., 2012) and calculated up to 80% TF_{max} to account for interindividual differences in the capability to exert force during the ramp contraction relative to their maximum (Mersmann et al., 2017b, 2018). This 80% TF_{max} was the highest common force relative to the individual maximum achieved by all participants. Tendon stiffness was calculated as slope of a linear regression between 50 and 80% TF_{max}. Tendon stress was calculated for the proximal and distal 40% of tendon length by dividing TF_{max} by the average tendon CSA of the respective section. Tendon elastic modulus was calculated as slope of a linear regression of the stress-strain relationship between 50 and 80% of maximum tendon stress, which was in this case calculated using the average CSA of the full tendon.

Assessment of Tendon Micromorphology

Tendon micromorphology was assessed based on a spatial frequency analysis of ultrasound images obtained at the proximal and distal part of the patellar tendon, respectively. The participants were positioned supine with the dominant leg flexed in the knee joint to 90° (0° = full extension), which was measured based on kinematic data captured with the Vicon system. The joint angle was chosen as, based on our experience, the tendon slack is removed and fascicles straightened, yet the passive joint forces are estimated to be as low as not to induce substantial strain (O'Brien et al., 2010). The linear transducer of an ultrasound system (My Lab60; Esaote, Genova, Italy; probe: linear array LA523, 13 MHz, depth: 3.0 cm) was placed over the patellar tendon parallel to its longitudinal axis below the most distal apex of the patella and the central aspect of the tibial tuberosity, respectively.

In the analysis of the captured images a polygonal region of interest (ROI) was defined in a custom written MATLAB interface (version R2016a; MathWorks, Natick, MA, United States). The length of the ROI corresponded to 40% of the tendon rest length of each participant (measured as described below) and its height covering the full thickness of the tendon. The location of the ROI was chosen that it spanned from the deep insertion to the central portion of the tendon, respectively. Within this ROI, as many kernels of 32 × 32 pixels as possible were analyzed as suggested by Bashford et al. (2008), by applying a 2D fast Fourier transform (FFT) and highpass filter with a radial frequency response and half-power cutoff frequency of 1.23 mm^{-1}. The filtered kernels were zero-padded in both directions to a size of 128 × 128 pixels and the distance of the peak spatial frequency (PSF) from the spectral origin in the frequency spectrum was used as measure of the packing density and alignment of the collagen bundles (Bashford et al., 2008). See **Figure 2A** for an illustration of the procedure.

Assessment of Tendon Neovascularization

A color Doppler examination to determine intratendinous blood flow was performed with the participants laying supine with the dominant knee flexed to 30° (0° = full extension). The joint angle was chosen based on former recommendations (Giombini et al., 2013; Bode et al., 2017) to reduce slack but avoid any passive forces that might induce vascular compression. The ultrasound system, probe model and orientation were as described above, yet the system settings were set as follows: CFM mode, 13 MHz frequency, 750 Hz peak repetition frequency, 1.1 by 1.6 cm color box size (spanning from the deep insertion at the patella or tibial tuberosity to the central portion of the tendon, respectively). The color gain was adjusted individually just below noise level. The probe pressure was kept to a minimum to avoid vascular obliteration. The tendon was scanned carefully in its full width moving the probe stepwise medio-laterally while keeping its longitudinal axis aligned with the longitudinal axis of the tendon. A short video sequence (i.e., ∼2–3 s) was captured at the site with the approximate strongest doppler signal. In the analysis, a polygonial ROI representing the tendon body within the color box was selected in the custom written MATLAB interface and the color area of the video frames were calculated based on the sum of all colored pixels in the ROI (see also **Figure 2B**). The largest color area of the sequence was used in the statistical evaluation.

Statistical Analysis

Normality of the data was analyzed using the Shapiro–Wilk test. In case of normality, correlations between the main outcome parameters were calculated using the Pearson correlation coefficient (r) or else using Spearman's rho (ρ). All statistical tests were run in SPSS (version 25, IBM, Armonk, NY, United States) with the alpha level set to 0.05.

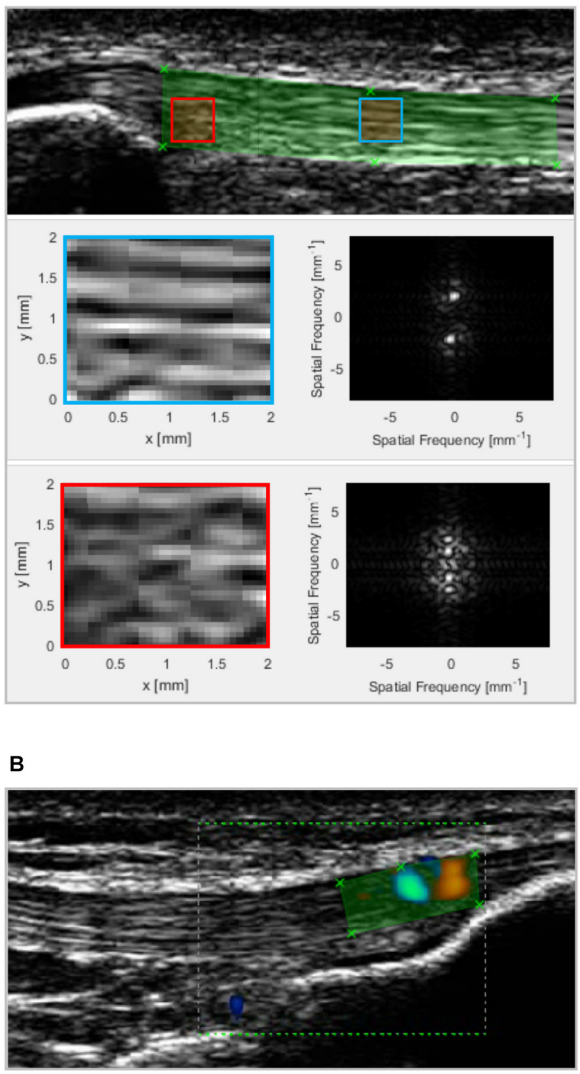

FIGURE 2 | (A) Patellar tendon micromorphology was assessed by applying a spatial frequency analysis on ultrasound images obtained from the proximal (shown) and distal tendon. All possible 32 × 32 pixel kernels within a polygonal region of interest (ROI; green) were filtered and 2D fast Fourier transformed. The blue and red squares represent two kernels with a high and low degree of fascicle packing and alignment and, thus, peak spatial frequency (2.2 and 1.4 mm^{-1}, respectively). The panels on the right to the enlarged kernels show the respective frequency spectrum. The average PSF value of all kernels was used in the statistical analysis. **(B)** Vascularisation was quantified by measuring the color area within a polygonal ROI that covered all intratendinous color doppler signals (exemplary distal image shown).

RESULTS

The main outcome parameters of patellar tendon morphological and mechanical are presented in **Table 1**. Mean ± standard deviation of proximal and distal tendon PSF and color doppler area was 1.78 ± 0.25, 1.91 ± 0.13 mm^{-1} and 2.21 ± 4.11, 6.52 ± 5.89 mm^2, respectively. The average VISA-P score of the affected participants (n = 6) was 73.8 ± 6.5 and for the unaffected 100 ± 0. There was a significant correlation of tendon strain and proximal tendon PSF (r = −0.652, p = 0.006; **Figure 3**). However, no significant association was found between strain and distal tendon PSF (r = 0.279, p = 0.3) or between tendon force (proximal: r = −0.24, p = 0.37; distal: r = −0.348, p = 0.19) or stress (proximal: ρ = −0.344, p = 0.19; distal: ρ = −0.068, p = 0.8) with tendon PSF at both sites, respectively (**Figure 3**). No significant correlations were observed between color doppler area and tendon mechanical parameters (ρ ranging from −0.17 to 0.08; $p \geq 0.54$).

Normalized tendon stiffness correlated significantly with tendon force (r = 0.704, p = 0.002) but no significant correlation was found for tendon elastic modulus and average tendon PSF (ρ = −0.179, p = 0.506; **Figure 4**).

TABLE 1 | Descriptive statistics of the patellar tendon morphological and mechanical properties of the investigated adolescent basketball athletes (n = 16).

	Mean ± SD
Tendon moment arm [mm]	54.1 ± 3.7
Tendon rest length [mm]	52.9 ± 6.8
Proximal tendon CSA [cm^2]	1.18 ± 0.20
Distal tendon CSA [cm^2]	1.19 ± 0.21
Tendon force [N]	4399 ± 898
Tendon stiffness [N/mm]	1338 ± 422
Normalized tendon stiffness [kN/strain]	73.1 ± 22.0
Proximal tendon stress [MPa]	38.7 ± 13.2
Distal tendon stress [MPa]	38.6 ± 13.3
Tendon strain [%]	6.6 ± 1.2

CSA: cross-sectional area.

DISCUSSION

The present study investigated the association of patellar tendon loading (in terms of force, stress, and strain) and structural characteristics of the tendon in adolescent basketball athletes. We found a significant inverse correlation between tendon strain and proximal tendon PSF, suggesting that tendons that are subjected to a higher mechanical demand show lower levels of collagen fascicle organization. This was not found at the distal tendon site and no significant correlations were observed between tendon force or stress and tendon PSF as well as between tendon loading and vascularity. Finally, normalized tendon stiffness was closely associated to tendon force, yet, against our assumptions, the tendon elastic modulus was not associated with the measure of micromorphology (i.e., PSF). Therefore, our hypotheses were only partly confirmed.

Tendons commonly adapt to the force generating capacity of their associated muscles to maintain tendon strain during loading in a physiological range (LaCroix et al., 2013). Therefore, a close association of tendon force and stiffness has been reported earlier (Scott and Loeb, 1995; Arampatzis et al., 2007b; Waugh et al., 2011; Mersmann et al., 2016) and was confirmed for adolescent basketball athletes in the present study. However, within the course of a training process, imbalances in the adaptation of muscle and tendon can cause episodes of increased

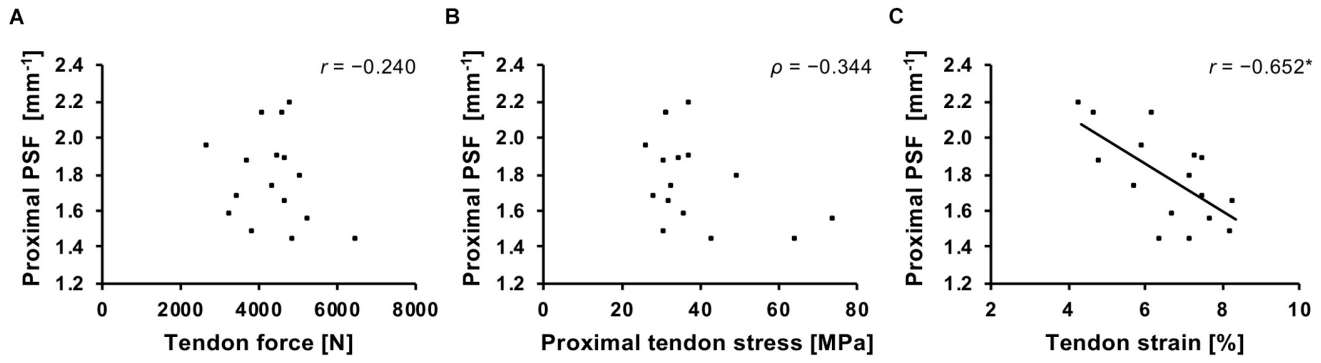

FIGURE 3 | Association of patellar tendon force **(A)**, stress **(B)**, and strain **(C)** with the peak spatial frequency (PSF) of the proximal tendon. *r*, Pearson correlation coefficient; ρ, Spearman's rho. *$p = 0.006$.

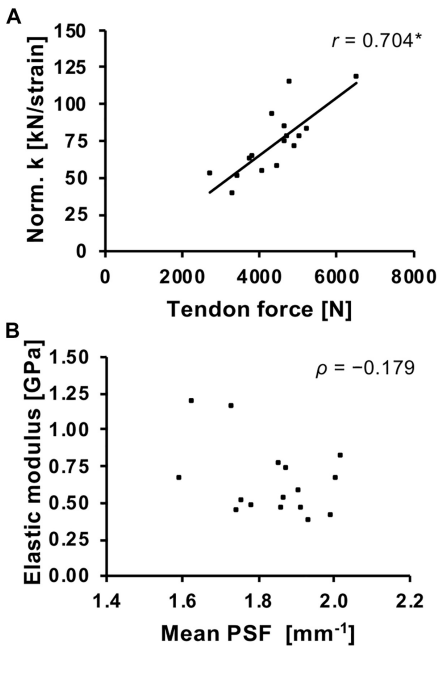

FIGURE 4 | Association of patellar tendon force and normalized tendon stiffness (Norm. k; **A**) and average tendon PSF and elastic modulus **(B)**. *r*, Pearson correlation coefficient; ρ, Spearman's rho. *$p < 0.01$.

tendon strain (Mersmann et al., 2016). In the present study, the average tendon strain at 80% of the maximum tendon force was 6.6%, which approximates to 7.9% (ranging from 5.2 to 9.6%) for a maximum muscle contraction when extrapolated based on tendon stiffness. This compares well to what we observed earlier in adolescent volleyball athletes (Mersmann et al., 2016, 2017b), which demonstrated significantly higher levels of strain (7.6–8.0%) compared to similar-aged controls (5.5–6.4%). Repeated exposure to high levels of tendon strain might harm the integrity of the tendinous tissue and increase the risk of injury (Wren et al., 2003; Fung et al., 2009). In the present study, we found a significant inverse relationship of the patellar tendon strain during isometric contractions and the proximal tendon PSF, which estimates the level of organization of the collagenous scaffold. Low values of PSF correspond to a less compacted and more isotropic speckle pattern of the ultrasound images, which is characteristic for tendinopathic tendons (Bashford et al., 2008), likely due to increased water content and collagen disorganization (Xu and Murrell, 2008; Magnusson et al., 2010). Therefore, our data can be interpreted as evidence for structural degeneration in the patellar tendons of those adolescent basketball athletes that are subjected to high levels of strain, while peak force or stress show no direct association with structural degeneration.

Indications for tendon matrix disorganization were present only at the proximal tendon site, which is also the most common site of pain and histological abnormalities (Roels et al., 1978; Ferretti et al., 1983). Lavagnino et al. (2008) further predicted greatest localized tendon strains at this region in a finite element model and found fascicle disruption at this location in their model-validation with cadaveric specimen. The finding is also in line with a cross-sectional study reporting lower proximal PSF values in symptomatic athletes compared to asymptomatic athletes and untrained controls, with no differences at the distal tendon (Kulig et al., 2013). In our study, we also assessed tendon pain at the time of data acquisition and again 2 month later using the VISA-P questionnaire. When clustering the participants of the present study to a group of asymptomatic athletes (reporting either no pain or recovering from tendinopathy to a VISA-P score of $\geq$ 85 after 2 months; $n = 10$) or symptomatic athletes (reporting pain on both occasions or developing tendinopathy within the 2 months; $n = 6$), we found significantly higher strain and lower proximal tendon PSF in the symptomatic group (Welch's *t*-test $p = 0.04$ and 0.02, respectively; **Figure 5**). The two recovering participants that were reporting pain only in the first session and were included in the asymptomatic group did not show any indications of structural degeneration (PSF values of 1.87 and 1.88 mm^{-1}, respectively), which suggests that pain in tendinopathy can persist or occur irrespective of tissue damage as determined by means of ultrasound. Though these results might need confirmation using a larger sample, taken together, these findings provide a strong argument for the assumption that

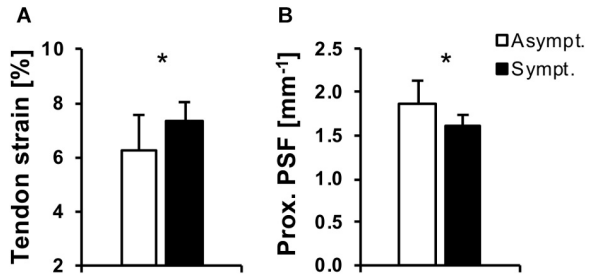

FIGURE 5 | Patellar tendon strain **(A)** and proximal tendon peak spatial frequency (PSF; **B**) in adolescent basketball athletes that were asymptomatic or recovering from tendinopathy (white) or had persistent symptoms or developed tendinopathy (black). *$p < 0.05$.

high tendon strain induced by an imbalance of muscle strength and tendon stiffness associates to structural degeneration of the tissue at the proximal patellar tendon and increases the risk for tendinopathy in adolescent athletes.

In our study, we did not find a significant correlation between average tendon PSF and elastic modulus, suggesting that the material properties of the tendon are not directly linked to the structural organization as quantified by the PSF. This was against our assumptions, since – though moderate in magnitude – such an association has been reported earlier in the Achilles tendon (Kulig et al., 2016). However, there is a remarkable variability of tendon material properties between and within species (LaCroix et al., 2013) and the elastic modulus is strongly influenced by the type and degree of intra- and inter-fibrillar cross-linking (Thompson and Czernuszka, 1995; Depalle et al., 2015; Lin and Gu, 2015), glycosaminoglycan content (Cribb and Scott, 1995) and collagen area fraction (Robinson et al., 2004). Therefore, it is well-comprehensible that the variation in material properties cannot be simply explained by the alignment of collagen fascicles. The discrepancy to the associations reported earlier by Kulig et al. (2016) might be partly be related to the use of a single cross-section obtained at the thickest part of the tendon in that study, which results in an underestimation of elastic modulus for the whole tendon, especially in individuals that show localized tendon swelling (and, thus, a low PSF). However, it might also be that the findings are specific to the tendon investigated (patellar vs. Achilles) and it might still be possible that changes in the structural organization as quantified by means of a PSF analysis of ultrasound images might associate not to baseline material properties but to changes of those due to pathogenesis or repair. In other words, a change in tendon PSF could reflect changes in the material properties, though the absolute elastic modulus might not be predicted only based on the structural appearance.

We did not find an association of tendon loading parameters and neovascularization. Further, the frequency of doppler findings was higher in the distal part of the tendon (75%) compared to the proximal site (31%), though degenerative processes are more common at the latter and also pain was exclusively reported to concern the proximal tendon in the symptomatic athletes. Intratendinous Doppler flow has often been linked to the presence or development of tendinopathy (McAuliffe et al., 2016). However, this view has also been challenged (Tol et al., 2012), since Doppler signals are actually quite common in asymptomatic athletes (Gisslén and Alfredson, 2005; Gisslèn et al., 2005; Boesen et al., 2006; Sengkerij et al., 2009). Cyclic loading of tendons stimulates the expression of endothelial growth factors, which mediate blood flow and the formation and ingrowth of new vessels, in a frequency-dependent manner (Petersen et al., 2004). Dynamic mechanical loading further seems to be able to induce a persistent increase of intratendinous blood volume (Kubo et al., 2009). Therefore, it has been speculated that in elite-level athletes the presence of Doppler flow in tendons is actually a physiological response associated to the high frequency of training (Malliaras et al., 2008; Koenig et al., 2010) and, thus, may not be necessarily indicative for degenerative processes or related to the level force, stress, or strain imposed on the tendon.

Considering the age of the athletes participating in the present study, individual differences in biological maturity might have influenced the structural appearance of the tendon. Rudavsky et al. (2017) reported a high variability of the echo patterns of the patellar tendons of young athletes before and around PHV and more consistent and continuous patterns similar to mature tendons after 1 year post-PHV. Such maturation-related structural changes would also affect the estimation of micromorphology as applied in the present study. The estimated PHV-offset of the athletes in the present study was 0.9 ± 0.7 years post-PHV, with half of the individuals to be characterized as peri-PHV (PHV-offset between -1 and $+1$ year) and the other post-PHV (offset ≥ 1 year). Though the more mature participants demonstrated higher tendon forces and elastic modulus ($r = 0.751$ and $\rho = 0.547$, respectively; $p < 0.05$), using PHV-offset as controlling variable in a partial correlation between tendon strain and proximal PSF did not change the main outcome ($r = -0.62$; $p = 0.014$). Therefore, we are confident that mechanical strain was the main determinant for the microstructural characteristics of the patellar tendon in our sample.

A limitation of the present study is the lack of a comparison to an untrained group and, to some extent, the combination of both healthy and affected athletes. Therefore, our conclusions regarding the association of tendon strain and microstructure remain confined to adolescent athletes, though it seems unlikely that such an association could be established in untrained adolescents, due to the quite consistent tendon strain during maximum contractions observed in earlier studies (Mersmann et al., 2016). Yet it seems promising to explicitly investigate the development of tendon mechanical and structural characteristics in groups of symptomatic and asymptomatic athletes to gain further insight into the etiology of tendinopathy.

CONCLUSION

The present study provides evidence that – in contrast to force or stress – high levels of tendon strain are associated to a micromorphological deterioration of the collagenous network in

the proximal patellar tendon of adolescent basketball athletes. Further, athletes suffering from or developing tendinopathy demonstrated both greater levels of tendon strain and lower levels of fascicle packing and alignment, which lends support to the idea that mechanical strain is the primary mechanical factor for tendon damage accumulation and the progression of overuse (Schechtman and Bader, 1997; Wren et al., 2003; Fung et al., 2009). Though still challenging from a methodological perspective (Seynnes et al., 2015; Mersmann et al., 2018), monitoring tendon strain in athletes might in perspective be a promising approach to assess tendon injury risk and then prescribe exercises targeting an increase in tendon stiffness for individuals that have an imbalance of muscle strength and tendon stiffness (Mersmann et al., 2017a). The assessment of tendon micromorphology might additionally be used to estimate the structural development of the tendon, with an analysis of the proximal tendon probably being sensitive for discriminating healthy and affected tissue. Tendon vascularity on the other hand seems unrelated to the mechanical demand and could be a physiological response to frequent training in adolescent elite athletes.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The participants and legal guardians gave written informed consent to the experimental procedures, which were approved by the ethics committee of the Humboldt-Universität zu Berlin (Ethikkommission der Kultur-, Sozial-, und Bildungswissenschaftlichen Fakultät) and carried out in accordance with the declaration of Helsinki.

AUTHOR CONTRIBUTIONS

FM and AA conceived the experiments, interpreted the data, and drafted the manuscript. FM, M-ST, and NP performed the experiments. FM and M-ST analyzed the data. AS and AA substantially contributed to data analysis. M-ST, NP, and AS made important intellectual contributions during revision. All authors approved the final version of the manuscript and agreed to be accountable for the content of the work.

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frontiers in Physiology

ORIGINAL RESEARCH
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Morphological and Mechanical Properties of the Quadriceps Femoris Muscle-Tendon Unit From Adolescence to Adulthood: Effects of Age and Athletic Training

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The combined effects of mechanical loading and maturation during adolescence are still not well understood. The purpose of the study was to investigate the development of the quadriceps femoris muscle-tendon unit from early adolescence (EA), late adolescence (LA) to young adulthood (YA), and examine how it is influenced by athletic training in a cross-sectional design. Forty-one male athletes and forty male non-athletes from three different age groups (EA: 12–14 years, n = 29; LA: 16–18 years, n = 27; and YA: 20–35 years, n = 25) participated in the present study. Maximum strength of the knee extensor muscles, architecture of the vastus lateralis (VL) muscle and patellar tendon stiffness were examined using dynamometry, motion capture, electromyography, and ultrasonography. Muscle strength and tendon stiffness significantly increased ($p < 0.001$) from EA to LA without any further alterations ($p > 0.05$) from LA to YA. Athletes compared to non-athletes showed significantly greater ($p < 0.001$) absolute muscle strength (EA: 3.52 ± 0.75 vs. 3.20 ± 0.42 Nm/kg; LA: 4.47 ± 0.61 vs. 3.83 ± 0.56 Nm/kg; and YA: 4.61 ± 0.55 vs. 3.60 ± 0.53), tendon stiffness (EA: 990 ± 317 vs. 814 ± 299 N/mm; LA: 1266 ± 275 vs. 1110 ± 255 N/mm; and YA: 1487 ± 354 vs. 1257 ± 328), and VL thickness (EA: 19.7 ± 3.2 vs. 16.2 ± 3.4 mm; LA: 23.0 ± 4.2 vs. 20.1 ± 3.3 mm; and YA: 25.5 ± 4.2 vs. 23.9 ± 3.9 mm). Athletes were more likely to reach strain magnitudes higher than 9% strain compared to non-athlete controls (EA: 28 vs. 15%; LA: 46 vs. 16%; and YA: 66 vs. 33%) indicating an increased mechanical demand for the tendon. Although the properties of the quadriceps femoris muscle-tendon unit are enhanced by athletic training, their development from early-adolescence to adulthood remain similar in athletes and non-athletes with the major alterations between early and LA. However, both age and athletic training was associated with a higher prevalence of imbalances within the muscle-tendon unit and a resultant increased mechanical demand for the patellar tendon.

Keywords: adolescent athletes, tendon stiffness, muscle strength, muscle architecture, muscle-tendon imbalances

INTRODUCTION

Human maturation describes the tempo and timing of the progress toward the mature state during growth (Mirwald et al., 2002). It is well known that during maturation the muscle-tendon unit is subjected to morphological and mechanical alterations (Kanehisa et al., 1995a; O'Brien et al., 2010; Kubo et al., 2014b). Muscle strength is increasing with age in line with body height and mass (Beunen and Malina, 1988; Kanehisa et al., 1995a; Degache et al., 2010), and increases markedly between 13 and 15 years in both sexes (Kanehisa et al., 1995a). Furthermore, Kanehisa et al. (Kanehisa et al., 1995a,b) reported an increase of the muscle anatomical cross-sectional area with age in parallel with muscle strength and, similarly, a pronounced development between age 13 and 15 years in untrained boys. The functional and morphological development of the muscle seems to continue until adulthood (Kubo et al., 2001, 2014b). On the other hand, there is evidence that the muscle strength in athletes increases most between 12 and 13 years in boys (Degache et al., 2010) and, thus, potentially earlier compared to untrained counterparts. Considering the increased secretion of muscle hypertrophy-mediating hormone levels, which occurs at that age (Vingren et al., 2010; Murray and Clayton, 2013) and is promoted by physical activity (Kraemer et al., 1992; Zakas et al., 1994; Tsolakis et al., 2004), it might even be that morphological changes of the muscle contribute to the adaptive response to increased mechanical loading. For instance, mid-adolescent athletes can already feature adult-like muscle morphology with only minor changes of muscle volume thereafter (Mersmann et al., 2014, 2017b) as well as greater muscle pennation angles compared to similar-aged controls (Mersmann et al., 2016). Thus, it seems possible that even early adolescent athletes already show indications of loading-related hypertrophy and muscle remodeling and that there is an interaction of maturation and superimposed loading that influences the temporal development of muscle during adolescence features (in terms of an earlier development) compared to untrained individuals.

Similar to muscles, tendon properties are also affected by the influence of maturation (O'Brien et al., 2009; Kubo et al., 2014b), including its cross-sectional area, Young's modulus (as a measure of its material properties based on the stress-strain relationship) and stiffness (as a measure of its mechanical resilience based on the force-elongation relationship). Tendon stiffness is a crucial mechanical property because it influences the transmission of the muscle force to the skeleton and depends on its material properties and dimensions (Butler et al., 1978). Patellar tendon stiffness and its determinants cross-sectional area (CSA), rest length and Young's modulus were reported to increase during maturation from 9 years to adulthood in humans (O'Brien et al., 2009). In accordance with the previous study, Kubo et al. (2014b) and Waugh et al. (2012) reported that Achilles tendon Young's modulus was lower in children (9–12 years) compared to adults, and junior high school students (13–15 years) had adult-like material properties. The mechanical changes observed from child- to adulthood may partly be mediated by an increase in the structural integrity of the collagenous network (Rudavsky et al., 2017, 2018). During pubertal growth, tendon length increases in a higher rate compared to the CSA, indicating that increments of tendon stiffness are mainly governed by a change of the material properties (Neugebauer and Hawkins, 2012; Waugh et al., 2012). Since tendons adapt to mechanical loading (Bohm et al., 2015), the increase of mass and muscle strength during maturation may increase the stiffness due to increased tendon loading during the daily weight-bearing tasks and the increased muscle force (Waugh et al., 2012). At the end of adolescence, tendon tissue turnover becomes greatly reduced (Heinemeier et al., 2013), yet the plasticity of the tendon is maintained, mainly in terms of loading-induced changes of the material properties (Bohm et al., 2015).

Irrespective of gains in body mass, superimposed mechanical loading by sports activity further can increase tendon stiffness in adolescence (Mersmann et al., 2017c), which suggests that the development of tendon mechanical properties during maturation might be different in athletes compared to adolescents that do not train systematically. Similar to muscle strength, data on the Achilles tendon of untrained adolescents suggest that the maturation-related increases of tendon stiffness are most pronounced early in adolescence (Kubo et al., 2014a; Mogi et al., 2018). Yet a study of our laboratory on adolescent volleyball athletes suggests that – under the twofold stimulus of maturation and training – major changes of tendon CSA and stiffness might occur later in adolescence compared to the muscular development (Mersmann et al., 2017b). Since there is little information considering muscle and tendon development during adolescence, there is still great uncertainty how maturation affects the muscle-tendon unit, especially in interaction with superimposed loading by means of athletic training. The increase of our understanding regarding this interplay might be of particular importance in terms of recent evidence, which lends support to the idea that an imbalanced development of muscle strength and tendon stiffness might increase the risk of overuse tendon injury (see Mersmann et al., 2017a for a review). An adequate strain applied to the tendon is important and necessary for tendon healthiness and adaptability (Bohm et al., 2015; Wiesinger et al., 2015). For example, mechanical tendon loading that introduce low strain values (∼3%) cannot improve tendon properties (Arampatzis et al., 2007a, 2010). However, if a tendon is repeatedly subjected to very high levels of strain, this might induce overload. In a rodent model, Wang et al. (2013) demonstrated that cyclic application of 9% tendon strain acts degenerative on the tissue and weakens its structural integrity. As ultimate tendon strain is irrespective of species (LaCroix et al., 2013) and considering the average levels of maximum *in vivo* tendon strain observed in humans using ultrasound (e.g., Hansen et al., 2006; Couppé et al., 2009; Mersmann et al., 2016, 2018), strain magnitudes higher than 9.0% during maximum isometric contractions might be indicative for imbalances within the muscle-tendon unit, characterized by the tendon stiffness being too low compared to the strength of the associated muscle (Bohm et al., 2019).

The purpose of this research was to investigate the musculotendinous development during adolescence and how

it is influenced by athletic training by means of comparing athletes and non-athletes in three different age groups (i.e., early adolescents: 12–14 years, late adolescents: 16–18 years and adults) under the reasonable assumption that these groups would also substantially differ in terms of maturation. We focused on the quadriceps femoris muscle-tendon unit due to its important contribution to movement performance and susceptibility to overuse injury (Zwerver et al., 2011; Simpson et al., 2016; Nikolaidou et al., 2017). We hypothesized to find higher muscle strength, muscle thickness, pennation angle, and tendon stiffness in athletes compared to non-athlete controls in all age groups. Moreover, we expected to find in athletes the major development of tendon stiffness between late adolescence (LA) and adulthood, yet more timely clear increases of muscle strength (Degache et al., 2010; Mersmann et al., 2017b), which may increase the mechanical demand for the tendon.

MATERIALS AND METHODS

Experimental Design

Eighty-one male participants comprised of athletes (n = 41) and untrained controls (n = 40) in three age groups [EA: early adolescence (n = 29), 12–14 years; LA: late adolescence (n = 27), 16–18 years; and YA: young adulthood (n = 25), 20–35 years] were included in the study (**Table 1**). The athletes were recruited from the disciplines American football, volleyball, handball, basketball, judo, kick-boxing, fencing, gymnastics, dancing, hockey, vaulting, track and field, acrobatics, decathlon, and trained at least three times per week for at least 75 min per session. Athletes from endurance sports were excluded, because the sport-specific low-intensity loading is unlikely to be a sufficient stimulus to significantly change the mechanical properties of the muscle-tendon unit (Karamanidis and Arampatzis, 2006; Arampatzis et al., 2007b). The sport activity of the untrained adolescent controls was limited to school sports and a maximum of one session of recreational sports per week, while in adults only the latter applied. None of the participants suffered from any orthopedic abnormality or injury at the lower extremities.

The study was carried out in accordance with the recommendations of the Ethics Committee of the Humboldt-Universität zu Berlin. All participants (and their respective legal guardians in the adolescent groups) gave written informed consent in accordance with the Declaration of Helsinki. The measurements of muscle strength (i.e., knee extension moments), vastus lateralis (VL) architecture and patellar tendon mechanical properties were carried out on the dominant leg (i.e., leg used for kicking a ball) following a standardized warm-up consisting of 2–3 min ergometer cycling, ten submaximal isometric contractions, and three maximum voluntary isometric contractions (MVC).

Measurement of Maximum Knee Joint Moment

For the assessment of the muscle strength of the knee extensor muscles, the participants performed isometric MVCs on a dynamometer (Biodex Medical System 3, Shirley, NY, United States) at 65°, 70°, and 75° knee joint angle (i.e., values at rest measured by the dynamometer; 0° = full knee extension). In our earlier work (e.g., Mersmann et al., 2017c), we found that using these resting angles, the participants reach their approximate optimum angle for force generation during the contractions. The trunk angle was set to 85° (neutral full hip extension = 0°) and the hip was fixed to the dynamometer seat using a non-elastic strap.

Since there are differences between the resultant knee joint moment and the moment measured by the dynamometer due to the changes of the knee joint axis relative to the axis of the dynamometer during the MVC induced by soft tissue deformation and dynamometer compliance, we followed the inverse dynamics approach introduced by Arampatzis et al. (2004). Kinematic data were recorded using a Vicon motion capture system (version 1.7.1; Vicon Motion Systems, Oxford, United Kingdom) integrating eight cameras operating at 250 Hz. Six reflective markers were captured, which were fixed on the following positions: lateral and medial malleolus, the most prominent points of the lateral and medial femoral condyles, trochanter major, and lateral aspect of the iliac spine. Passive knee joint moments due to gravity were recorded as a function of knee joint angle in an additional trial. The participants were instructed to relax the muscles of their dominant leg and then the joint was passively rotated at 5°/s through the full range of motion by the dynamometer. Further, we accounted for the contribution of antagonistic muscle activity to the resultant moment by establishing a linear electromyographic (EMG)-activity – knee flexion moment relationship during submaximal isometric contractions (Mademli et al., 2004). For this purpose,

TABLE 1 | Anthropometrical characteristics of the non-athletes and athletes in the three age groups (EA, early adolescence; LA, late adolescence; YA, young adulthood; means ± standard deviation).

	Non-athletes			Athletes		
	EA (n = 14)	LA (n = 13)	YA (n = 13)	EA (n = 15)	LA (n = 14)	YA (n = 12)
Age [years]	$12.8 \pm 0.6^{b,c}$	$17.3 \pm 0.8^{a,c}$	$29.0 \pm 3.6^{a,b}$	$13.0 \pm 0.8^{b,c}$	$17.2 \pm 0.8^{a,c}$	$26.3 \pm 3.0^{a,b}$
Body height [cm]*#	$159.6 \pm 11.0^{b,c}$	175.1 ± 5.3^{a}	179.4 ± 9.6^{a}	$168.6 \pm 12.0^{b,c}$	183.1 ± 8.4^{a}	182.1 ± 8.1^{a}
Body mass [kg]*	$45.4 \pm 10.3^{b,c}$	$70.1 \pm 15.0^{a,c}$	$80.7 \pm 16.5^{a,b}$	$56.2 \pm 11.2^{b,c}$	$72.7 \pm 10.4^{a,c}$	$79.5 \pm 9.1^{a,b}$
Femur length [cm]*#	$38.7 \pm 2.2^{b,c}$	41.0 ± 1.9^{a}	40.6 ± 3.8^{a}	$39.8 \pm 4.1^{b,c}$	43.8 ± 3.8^{a}	42.9 ± 2.9^{a}

#*Statistically significant effect of activity ($p < 0.05$).* **Statistically significant effect of age ($p < 0.05$).* [a]*Statistically significant difference to EA ($p < 0.05$).* [b]*Statistically significant difference to LA ($p < 0.05$).* [c]*Statistically significant difference to YA ($p < 0.05$).*

we recorded two additional knee flexion trials featuring an EMG-activity that was slightly lower and higher, respectively, compared to the activity registered during the maximum knee extension trials. The EMG activity of the lateral head of the biceps femoris was recorded using two bipolar surface electrodes (Blue Sensor N, Ambu GmbH, Bad Nauheim, Germany) placed over the mid-portion of the muscle belly with an inter-electrode distance of 2 cm after shaving and cleaning the skin to reduce skin impedance. EMG data was captured at 1000 Hz (Myon m320RX; Myon, Baar, Switzerland) and transmitted to the Vicon system via a 16-channel A-D converter.

Measurement of Vastus Lateralis Muscle Architecture

For the assessment of the VL architecture, ultrasound images were captured at 60° knee joint angle, which has been reported by Herzog et al. (1990) to be the approximate optimum angle of the VL for force production. A 10 cm linear ultrasound probe (7.5 MHz; My Lab60; Esaote, Genova, Italy; probe: linear array (LA923), depth: 7.4 cm, focal point: 0.9 and 1.9, no image filter) was placed over the belly of the inactive muscle in its longitudinal axis at 60% thigh length, which is the assumed location of the maximum anatomical cross-sectional area (Mersmann et al., 2015). The ultrasound images were analyzed offline using a custom written MATLAB interface (version R2012a; MathWorks, Natick, MA, United States). The upper and deeper aponeuroses were defined by setting three reference points along each aponeurosis and a linear least-squares-fit through these points. Subsequently, the visible features of multiple fascicles were marked manually and a reference fascicle was calculated based on the average inclination of the fascicle portions and the distance of the aponeuroses (Marzilger et al., 2017). The pennation angle refers to the angle between the reference fascicle and the deeper aponeurosis. Fascicle length was normalized to femur length (measured from the greater trochanter to the lateral epicondyle, identified by palpation, by means of a measuring tape).

Mechanical Properties of the Patellar Tendon

To investigate the force-elongation relationship of the patellar tendon, the ultrasound probe (i.e., similar probe and settings as described previous) was fixed by means of a custom-made knee brace overlying the patellar tendon in the sagittal plane. The participants performed 5 isometric ramp contractions, gradually increasing their effort from rest to maximum in ∼5 s and simultaneously the elongation of the tendon was captured by means of the ultrasound at 25 Hz. The resting knee joint angle for the ramp contractions was set according to the MVC trial in which the highest moment was achieved by the respective participant. The knee joint moments were calculated according the same consideration as described above, applying the inverse dynamics approach and correction for antagonistic activity. Tendon force was calculated by dividing the knee extension moment by the tendon moment arm.

The moment arms were predicted using the regression equation reported by Mersmann et al. (2016) based on sex, body height, and mass. Since the moment arm of the patellar tendon is significantly influenced by the knee joint angle, it was adjusted to the respective knee joint angle position based on the polynomial regression equation suggested by Herzog and Read (1993). The ultrasound images were synchronized with the kinematic and analog data using an externally induced voltage peak. Patellar tendon elongation during the contractions was determined by manually tracking the deep insertion of the tendon at the patellar apex and the tibial tuberosity frame-by-frame using a custom-written MATLAB interface. In order to achieve a high reliability (≥0.95), the force-elongation relationship of the 5 trials of each participant was averaged using the highest common force of the single trials as a peak force (Schulze et al., 2012). Tendon stiffness was calculated between 50 and 100% of the peak tendon force based on a linear regression. As stiffness is influenced by the resting length of the tendon (Butler et al., 1978; Arampatzis et al., 2005), we further calculated the normalized tendon stiffness (i.e., the product of stiffness and rest length) that represents the slope of the force-strain curve.

STATISTICS

The statistical analysis was conducted in SPSS (version 20.0; IBM, Armonk, NY, United States). A two–way analysis of variance (ANOVA) was performed with the fixed factors activity (i.e., non-athletes, athletes) and age (i.e., EA, LA, and YA) The Shapiro–Wilk Test was performed to verify the normal distribution of the data and Levene's test to assess the homogeneity of variances. A Bonferroni-corrected *post hoc* analysis was conducted in the case of a significant age effect or interaction of the factors activity and age. The alpha level for all tests was set to 0.05. The effect size f for significant observations were calculated in G*Power (Version 3.1.6; HHU, Düsseldorf, Germany; Faul et al., 2007), based on the partial eta squared or means and pooled standard deviation for non-parametrically tested parameters. The subscript *Activity* and *Age* indicates if the effect size refers to differences between athletes and controls or between age groups, respectively. Effect sizes of $0.1 \leq f < 0.25$ will be referred to as small, $0.25 \leq f < 0.5$ as medium and $f \geq 0.5$ as large (Cohen, 1988). Using the whole sample, we calculated the Pearson's r for the correlation of tendon force and stiffness. We further predicted tendon stiffness by tendon force using a linear regression model with group-specific y-intercept and slope constants for each age and activity group, respectively, and compared the residuals of the model prediction with a two-way ANOVA to analyze differences in the association of tendon force and stiffness. The model equation was:

$$y_i = c_0 + \beta_0 F_i + c_1 g_i + \beta_1 g_i F_i + c_2 l_i + \beta_2 l F_i + c_3 g_i l_i + \beta_3 g_i l_i F_i + c_4 a_i + \beta_4 a_i F_i + c_5 g_i a_i + \beta_5 g_i a_i F_i + \varepsilon_i$$

where i is index for participant (1,. . .,81); g is the activity-group variable (non-athlete = 0; athlete = 1); l is late adolescent age variable (EA = 0; LA = 1; YA = 0); a is young adult age variable (EA = 0; LA = 0; YA = 1); c are the intercept

constant, β are the slope constants; F is tendon force ε is the residual.

We further examined the frequency of individuals that reached strain values greater than 9%, since it has been reported that repetitive strains above 9% can induce catabolic tendon matrix damage (Wang et al., 2013). Though the exceedance of the threshold does not necessarily imply injury, it provides a classification if the mechanical demand for the tendon and risk for fatigue is comparatively high.

RESULTS

Considering the anthropometric data (**Table 1**), there was a significant effect of age on body mass ($p < 0.001$, $f_{Age} = 1.04$), but no effect of activity group or activity-by-age interaction ($p > 0.05$). *Post hoc* analysis revealed significantly greater body mass with increasing age of the respective group ($p < 0.05$). There was a significant effect of activity group ($p = 0.003$, $f_{Activity} = 0.36$) and age ($p < 0.001$, $f_{Age} = 0.83$) on body height. Athletes were taller compared to non-athlete controls and EA showed significantly smaller height compared to LA and YA ($p < 0.001$), but there were no significant differences between YA and LA ($p = 1.0$). There was a significant main effect of age and activity ($p = 0.002$, $f_{Age} = 0.43$; $p = 0.007$ $f_{Activity} = 0.32$, respectively) but no activity-by-age interaction ($p = 0.608$) on femur length. EA had smaller femur lengths compared to YA and LA ($p = 0.002$ and $p = 0.028$, respectively), but there were no significant differences between YA and LA ($p = 1.0$).

Considering absolute and normalized muscle strength (normalized to body mass) of the knee extensors, athletes had higher strength compare to non-athletes ($p < 0.001$, $f_{Activity} = 0.53$ for absolute strength and $p < 0.001$, $f_{Activity} = 0.59$ for normalized strength). There was a significant age effect ($p < 0.001$, $f_{Age} = 1.13$ for absolute strength, and $p < 0.001$, $f_{Age} = 0.64$ for normalized strength) but no activity-by-age interaction ($p = 0.770$ and $p = 0.129$ for the absolute and normalized strength, respectively; **Table 2**). EA had lower absolute strength compared to YA and LA ($p < 0.001$, $f = 1.14$, and $f = 0.93$, respectively) and normalized muscle strength ($p < 0.001$, $f = 0.51$, and $f = 0.61$), but there were no statistically significant differences between YA and LA ($p = 0.395$ and $p = 1.0$). There was no significant effect of age ($p = 0.743$), activity ($p = 0.370$) or activity-by-age interaction ($p = 0.532$ **Table 2**) on antagonistic co-activation (i.e., antagonistic moment normalized to maximal resultant moment) and tendon resting length ($p = 0.290$, $p = 0.930$, and $p = 0.505$, respectively). We found greater VL muscle thickness in athletes compared to non-athletes ($p = 0.001$, $f_{Activity} = 0.4$) and a significant effect of age ($p < 0.001$, $f_{Age} = 0.79$), but no effect of age-by-activity interaction ($p = 0.545$, **Figure 1A**). EA and LA had lower ($p < 0.001$, $f = 0.86$, and $p = 0.001$, $f = 0.48$) muscle thickness compared to YA, and EA lower thickness than LA ($p = 0.007$, $f = 0.41$). There was no effect of activity ($p = 0.473$) or age-by-activity interaction ($p = 0.407$) on pennation angle (**Figure 1B**). However, there was a significant effect of age ($p < 0.001$, $f_{Age} = 0.6$) on pennation angle (**Figure 1B**). EA, LA both had lower pennation angles compared to YA ($p < 0.001$, $f = 0.65$, and $p = 0.001$, $f = 0.51$), but there were no statistically significant differences between EA and LA ($p = 0.707$). On normalized fascicle length (normalized to femur length), there were no significant effects of age ($p = 0.903$), activity ($p = 0.299$) or age-by-activity interaction ($p = 0.935$; **Figure 1C**).

Patellar tendon maximal force was greater in athletes compared to non-athletes ($p < 0.001$, $f_{Activity} = 0.52$) and there was a significant effect of age ($p < 0.001$, $f_{Age} = 1.12$), but no significant age-by-activity interaction ($p = 0.772$, **Figure 2A**). EA had significant smaller patellar tendon force compared to LA and YA ($p < 0.001$, $f = 0.93$, and $f = 1.13$, respectively), but there were no significant differences between LA and YA ($p = 0.602$). Athletes had stiffer patellar tendons compared to non-athletes ($p = 0.013$, $f_{Activity} = 0.31$, **Figure 2B**) and there was a significant effect of age ($p < 0.001$, $f_{Age} = 0.61$). EA had statistically lower patellar tendon stiffness compared to YA ($p = 0.015$, $f = 0.66$) and LA ($p < 0.001$, $f = 0.42$), but there were no significant differences between YA and LA ($p = 0.104$). There was a significant effect of age ($p < 0.001$, $f_{Age} = 0.66$) and a significant effect of activity ($p = 0.01$, $f_{Activity} = 0.32$) on normalized patellar tendon stiffness (**Table 2**), but no statistically significant activity-by-age interaction ($p = 0.956$). EA had smaller normalized patellar tendon stiffness compared to LA ($p = 0.001$, $f = 0.55$) and YA ($p < 0.001$, $f = 0.70$) but no significant differences between LA and YA ($p = 0.592$). There was a significant effect of age on patellar tendon maximum strain ($p = 0.028$, $f_{Age} = 0.33$; **Figure 2C**). EA

TABLE 2 | Knee joint moments, co-activation (i.e., antagonistic moment normalized to the resultant knee joint moment), tendon resting length, and normalized stiffness of the non-athletes and athletes in the three age groups (EA, early adolescence; LA, late adolescence; YA, young adulthood; means ± standard deviation).

	Non-athletes			Athletes		
	EA ($n = 14$)	LA ($n = 13$)	YA ($n = 13$)	EA ($n = 15$)	LA ($n = 14$)	YA ($n = 12$)
MVC [Nm]*#	145.2 ± 34.6[b,c]	267.0 ± 72.3[a]	288.2 ± 61.0[a]	202.0 ± 65.7[b,c]	327.3 ± 69.4[a]	367.0 ± 64.7[a]
Normalized MVC [Nm/kg]*#	3.20 ± 0.42[b,c]	3.83 ± 0.56[a]	3.60 ± 0.53[a]	3.52 ± 0.75[b,c]	4.47 ± 0.61[a]	4.61 ± 0.55[a]
Antagonistic co-activation [%]	8.4 ± 4.3	11.1 ± 6.3	8.9 ± 6.1	8.5 ± 6.3	8.1 ± 5.1	8.5 ± 4.5
Tendon resting length [mm]	49.2 ± 8.5	52.0 ± 4.4	51.0 ± 8.4	50.6 ± 6.9	53.0 ± 7.6	48.1 ± 5.9
Tendon normalized stiffness [kN/strain]*#	41.5 ± 11.6[b,c]	57.2 ± 11.1[a]	63.3 ± 15.7[a]	51.0 ± 15.1[b,c]	65.9 ± 14.7[a]	70.5 ± 14.5[a]

*#Statistically significant effect of activity ($p < 0.05$). *Statistically significant effect of age ($p < 0.05$). [a]Statistically significant difference to EA ($p < 0.05$). [b]Statistically significant difference to LA ($p < 0.05$). [c]Statistically significant difference to YA ($p < 0.05$).*

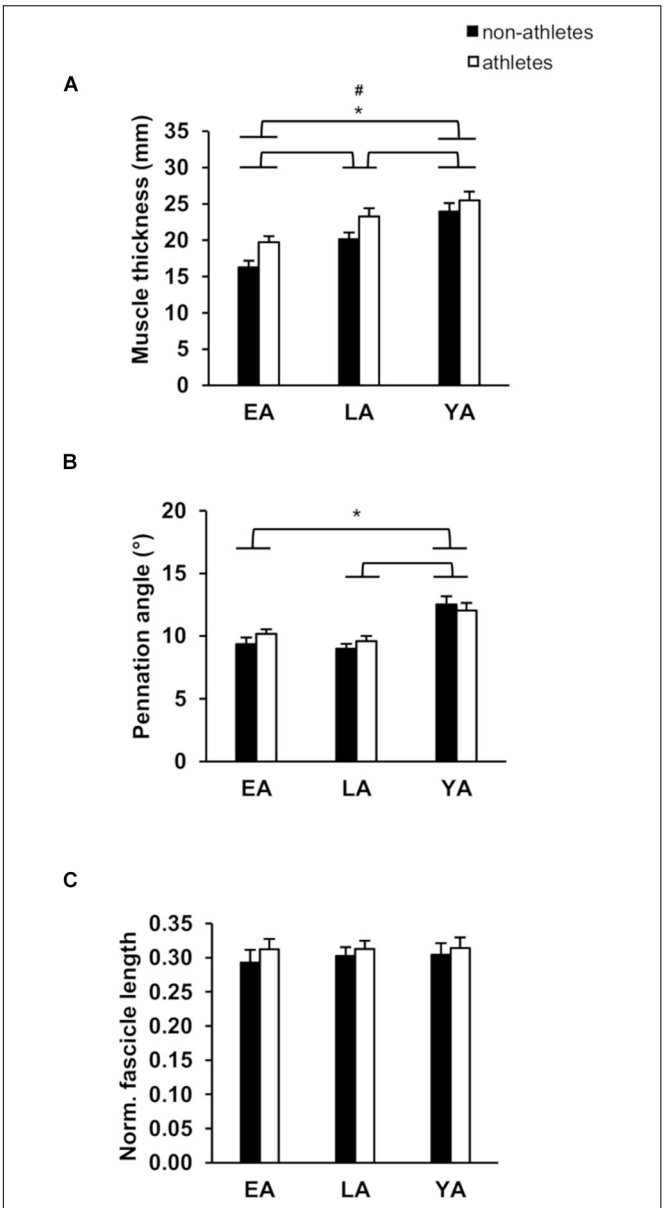

FIGURE 1 | Mean values and standard error (error bars) of vastus lateralis (VL) muscle thickness **(A)**, pennation angle **(B)**, and normalized fascicle length (**C**; normalized to femur length) of non-athletes (black) and athletes (white) in early adolescence (EA), late adolescence (LA), and young adulthood (YA). #Statistically significant effect of activity ($p < 0.05$). *Statistically significant effect of age ($p < 0.05$).

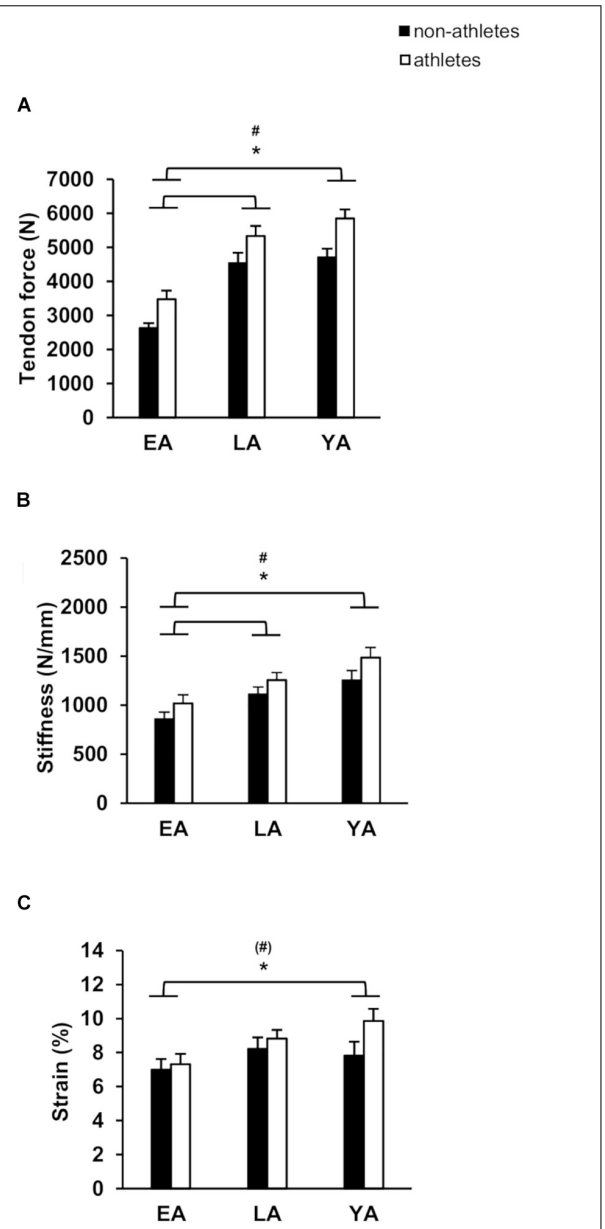

FIGURE 2 | Mean values and standard error (error bars) of patellar tendon: tendon force **(A)**, tendon stiffness **(B)**, and tendon strain **(C)** of non-athletes (black) and athletes (white) in EA, LA, and YA. #Statistically significant effect of activity ($p < 0.05$). *Statistically significant effect of age ($p < 0.05$). (#)Tendency for an effect of activity, $p = 0.072$.

had lower tendon strain compared to YA ($p = 0.039$, $f = 0.33$), but there were no statistically significant differences between EA and LA ($p = 0.120$), or LA and YA ($p = 1.0$). There was a tendency toward an effect of activity on patellar tendon strain ($p = 0.072$, $f_{\text{Activity}} = 0.22$), but no age-by-activity interaction ($p = 0.389$).

There was a significant correlation between tendon force and tendon stiffness ($r = 0.631$, $p < 0.001$, **Figure 3A**) for the whole investigated group of participants. The residuals of the regression model that included group-specific terms showed a tendency for an activity effect ($p = 0.098$) and no effect of age ($p = 0.524$) or age-by-activity interaction (0.536, **Figure 3B**). Examining the individual tendon strain values during the maximum isometric contractions, it is notable that athletes were more likely to reach strain magnitudes higher than 9% strain compared to non-athlete controls (frequency in athletes: 28–66% and in non-athletes: 15–33%, **Figure 4**). Further, the frequency of individuals that reach strain values greater than 9% increased from EA to YA in both athletes and non-athletes (**Figure 4**).

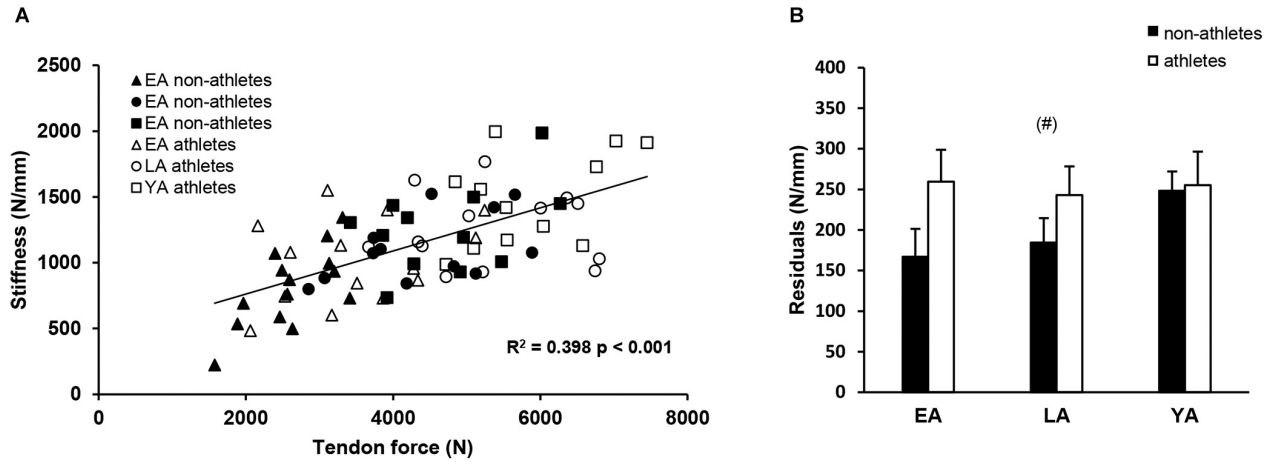

FIGURE 3 | (A) Correlation of tendon force and stiffness of non-athletes (black) and athletes (white) in early adolescence (EA, triangles), late adolescence (LA, circles), and young adulthood (YA, squares). **(B)** Means and standard error (error bars) of the residuals of the group-specific linear regression model (see section "Materials and Methods") of non-athletes and athletes in EA, LA, and YA. (#)Tendency for an effect of activity, $p = 0.098$.

DISCUSSION

The present cross-sectional study investigated the development of quadriceps femoris muscle strength, VL architecture and patellar tendon mechanical properties during adolescence and how it is influenced by athletic training. The results show that, both muscle and tendon were affected by athletic training, demonstrating greater muscle strength, tendon stiffness and VL thickness in athletes compared to non-athlete controls. However, although the absolute values were different between athletes and controls, the development of muscle strength, tendon stiffness and VL thickness from early adolescent to adulthood did not differ significantly, indicating a similar effect of maturation on muscle-tendon properties in both groups.

There was a marked increase in muscle strength of the knee extensors from early to late adolescents in both athletes (62%) and non-athletes (86%) and no differences between late adolescent and young adults. These findings are in agreement with earlier studies reporting the effect of maturation on the muscle strength development (Kanehisa et al., 1995a,b; Landi et al., 2017). In all investigated age-groups, athletes demonstrated greater muscle strength and VL muscle thickness compared to non-athletes, evidencing a training-induced adaptation in the knee extensor muscles. Furthermore, the increased VL muscle thickness indicates muscle hypertrophy even in the EA as a consequence of intensive athletic training. Similarly, a marked increase from EA to LA without any differences between LA and YA and a clear effect of athletic training was found in patellar tendon stiffness and normalized patellar tendon stiffness. In a previous study (Mersmann et al., 2017c), we reported greater patellar tendon stiffness in late-adolescent Volleyball athletes compared to untrained controls, demonstrating the tendon's responsiveness to mechanical loading in this age. Our current study provides additional evidence that tendons adapt to increased mechanical loading and enhance their stiffness already in EA. In our EA participants, the average enhancement in patellar tendon stiffness due to training was ∼25%, which can be interpreted as clear and functionally relevant adaptation.

The main alteration in muscle strength and tendon stiffness due to maturation seems to occur between EA and LA. In this stage also the main changes in the femur length, body height and body mass occurred, which indicates an analogous development of the functional and mechanical muscle-tendon properties with the skeletal system. Further, normalized fascicle length (fascicle length/femur length) was similar between all age groups and without any athletic training effect, indicating that during maturation fascicle length development is proportional to bone growth. To our knowledge, this is the first study investigating the interaction between athletic training and age in both muscle and tendon properties during adolescence. We hypothesized an effect of athletic training on the development in muscle and tendon properties during adolescence because the level of the androgenic hormones (e.g., testosterone), which promote protein synthesis and, thus, muscle hypertrophy (Murray and Clayton, 2013; Lundberg, 2017), is different in each stage of maturation and can additionally be affected by athletic training (Kraemer et al., 1992; Zakas et al., 1994; Tsolakis et al., 2004). The absence of any age-by-activity interaction indicates that, irrespective of the marked differences in the average levels of muscle strength and tendon stiffness, the course of the development of these muscle-tendon unit properties with maturation is similar in athletes compared to non-athletes. This is somewhat in contrast to our earlier assumptions (Mersmann et al., 2017a) and the conclusion of earlier meta-analyses (Behringer et al., 2010; Moran et al., 2017) that the trainability of muscle strength and the anabolic response of muscles to mechanical stimuli would increase during adolescent maturation, which we thought would affect the course of muscle-tendon development with increasing differences between the athletes and controls with age. Though the systemic basal levels of sex and growth hormones

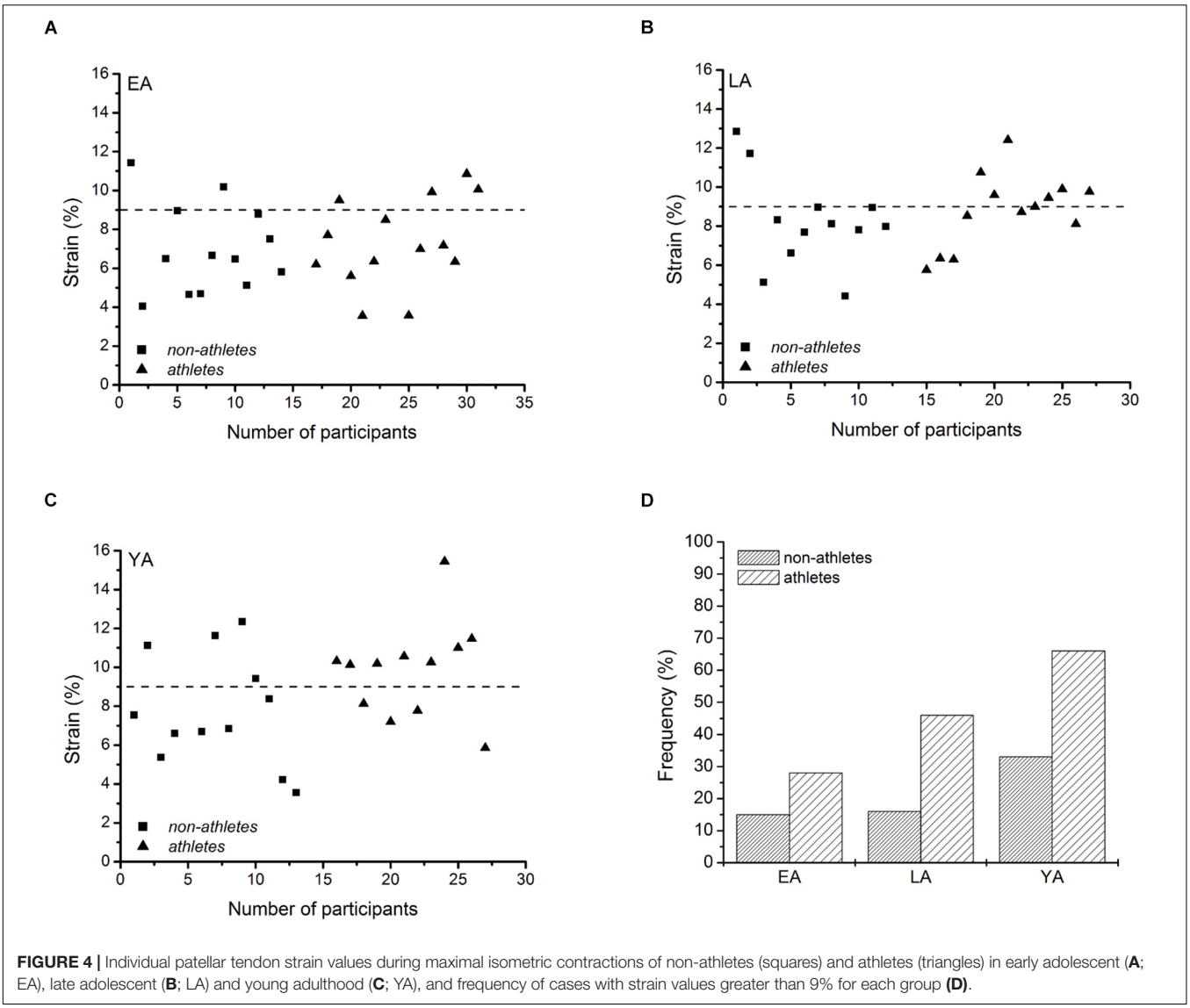

FIGURE 4 | Individual patellar tendon strain values during maximal isometric contractions of non-athletes (squares) and athletes (triangles) in early adolescent (**A**; EA), late adolescent (**B**; LA) and young adulthood (**C**; YA), and frequency of cases with strain values greater than 9% for each group **(D)**.

(Murray and Clayton, 2013) and the endocrine response to exercise increase with maturation and influence muscle and tendon protein metabolism (Rooyackers and Nair, 1997; Hulthén et al., 2001; Doessing et al., 2010; Hansen and Kjaer, 2014), the local responses of the muscle-tendon unit to training seems not to be a simple function of the maturation-related changes of the basal levels and load-induced secretion of systemic hormones. For instance, research that directly compared the effects of training in states of high or low concentrations of circulating endogenous hormones found no differences in the intramuscular anabolic signaling (Spiering et al., 2008), acute protein synthesis (West et al., 2009), or the local functional and morphological response to repeated training sessions (West et al., 2010).

In our study, we found an effect of age on tendon strain during maximum contractions with significantly higher tendon strain in adults compared to EA, indicating a disproportionate increase of tendon force compared to stiffness with increasing age. Further, although statistically not significant, the strain values during the maximum isometric contractions as well as the residuals of the regression model predicting tendon stiffness by tendon force were in tendency greater in athletes ($p = 0.072$ and $p = 0.098$, respectively). When examining the individual strain values reached during the maximum isometric contractions in all investigated age groups, it is notable that it was more likely in athletes that individuals reached strain magnitudes higher than 9%, which is indicative of imbalances within the muscle-tendon unit and resultant high mechanical demand for the tendon. Further, the frequency of strain values over 9% increased from EA to YA independent of activity status. These observations lend support to the idea that both athletic training and maturation can lead to an increased prevalence of imbalances between muscle strength and tendon stiffness. Several studies (Lian et al., 2005; Zwerver et al., 2011; Cassel et al., 2015; Simpson et al., 2016) reported a similar phenomenon for the prevalence of tendinopathy with regard to maturation and athletic training (i.e., increased prevalence from EA to YA and in athletes). An

increase of overall tendon strain has been shown to increase local tissue strains at the common site of structural degeneration in patellar tendinopathy (Lavagnino et al., 2008). Further, we recently found an association of tendon strain and its structural integrity in adolescent basketball players as well as increased strain and impaired tendon microstructure in a subgroup with tendinopathy (Mersmann et al., 2019). Thus, imbalances between muscle strength and tendon stiffness developing during maturation and with athletic training, repetitively subjecting the tendon to high levels of strain, might be a risk factor in the etiology of overuse-induced tendinopathy as well as the common background tendinosis or the, rather rare, tendinitis. Further, one might speculate that an increase of tendon strain during muscle contraction might lead to a maltracking of the patellar, redistribution of loads at the patellofemoral contact area and, in consequence, patellofemoral pain (Powers et al., 2017), which is also common in adolescents (Rathleff, 2016). Though the association of musculotendinous imbalances to mechanisms of overuse injury warrants experimental evidence, from a preventive point of view, the integration of a specific training that increases tendon stiffness and facilitates a balanced adaptation between muscle and tendon might be an important approach for the athletic practice. Previous research of our group indicates that an effective training stimulus for tendon adaptation is a combination of high loading magnitude, an appropriate loading duration in every repetition (i.e., 3 s) and repetitive loading (Arampatzis et al., 2007a, 2010; Bohm et al., 2014). In children, the development of resistance training competency should precede the application of high loads (Lloyd et al., 2014), yet it has already been shown that specific tendon training in accordance to the exercise recommendations above can be successfully applied in children to increase their tendon stiffness (Waugh et al., 2014). A more comprehensive discussion of tendon training in children and adolescents for the prevention of muscle-tendon imbalances and tendinopathy and specific exercise recommendations can be found elsewhere (Mersmann et al., 2017c).

A limitation of the present study is the lack of control for biological age. However, the assessment of skeletal age involves exposure to radiation and, in addition to the perceived invasiveness, the accuracy of grading the secondary sex characteristics is rather low (Schlossberger et al., 1992; Taylor et al., 2001; Slough et al., 2013), which is a particular problem for small sample comparisons. Estimations of maturity based on anthropometric data are a tempting alternative, yet these predictions cannot account for the considerable variation in anthropometry at a similar stage of maturity. As we included athletes from sports in which body height is a selection criterion (e.g., basketball and volleyball) and, as a result, our athletes were significantly taller compared to the non-athlete controls, any anthropometry-based prediction would also suggest a higher level of maturity in athletes. Even if that might not reflect actual differences in biological age, we cannot rule out differences in maturity. While maturity-related differences in physical characteristics have been reported to be largely eliminated in non-athletes and athletes aged 16–18 (Malina et al., 2004, 2013), the differences observed between athletes, and non-athletes need to be interpreted with care considering the EA group. On the other hand, it seems very unlikely that the clear differences in calendric age between age-groups would not be representative for different stages of maturity. Therefore, we do not believe that our conclusions considering the effects of maturation are affected by the lack of an assessment of actual maturity. Finally, due to the inherent limitations of cross-sectional studies, further longitudinal research is needed to confirm the development of the musculotendinous system and its interaction with mechanical loading indicated by our data.

CONCLUSION

In conclusion, the present study provides evidence that aside from higher levels of muscle strength, muscle thickness and tendon stiffness in athletes, the development of the properties of the knee extensor muscle-tendon unit from early-adolescence to adulthood is similar in athletes and non-athlete controls, with the major alterations occurring between early and LA. The frequency of imbalances in the quadriceps femoris muscle-tendon unit seem to increase with both age and athletic training during the adolescence-to-adulthood development and result in an increased mechanical demand for the patellar tendon. Therefore, we recommend to introduce specific intervention protocols in the athletic training practice in order to support a balanced adaptation between muscle and tendon.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Ethics Committee of the Humboldt-Universität zu Berlin. All participants (and their respective legal guardians in the adolescent groups) gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

GC and AA conceived the experiments. GC, FM, and SB performed the experiments. GC analyzed the data. FM, SB, and AA substantially contributed to the data analysis. GC, FM, and AA interpreted the data and drafted the manuscript. SB made important intellectual contributions during revision. All authors approved the final version of the manuscript and agreed to be accountable for the content of the work.

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Calcaneal Tendon Plasticity Following Gastrocnemius Muscle Injury in Rat

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Cross-talk between skeletal muscle and tendon is important for tissue homeostasis. Whereas the skeletal muscle response to tendon injury has been well-studied, to the best of our knowledge the tendon response to skeletal muscle injury has been neglected. Thus, we investigated calcaneal tendon extracellular matrix (ECM) remodeling after gastrocnemius muscle injury using a rat model. Wistar rats were randomly divided into four groups: control group (C; animals that were not exposed to muscle injury) and harvested at different time points post gastrocnemius muscle injury (3, 14, and 28 days) for gene expression, morphological, and biomechanical analyses. At 3 days post injury, we observed mRNA-level dysregulation of signaling pathways associated with collagen I accompanied with disrupted biomechanical properties. At 14 days post injury, we found reduced collagen content histologically accompanied by invasion of blood vessels into the tendon proper and an abundance of peritendinous sheath cells. Finally, at 28 days post injury, there were signs of recovery at the gene expression level including upregulation of transcription factors related to ECM synthesis, remodeling, and repair. At this time point, tendons also presented with increased peritendinous sheath cells, decreased adipose cells, higher Young's modulus, and lower strain to failure compared to the uninjured controls and all post injury time points. In summary, we demonstrate that the calcaneal tendon undergoes extensive ECM remodeling in response to gastrocnemius muscle injury leading to altered functional properties in a rat model. Tendon plasticity in response to skeletal muscle injury merits further investigation to understand its physiological relevance and potential clinical implications.

Keywords: muscle-tendon interaction, tendon disorders, tenocyte, muscle damage, calcaneal tendon

INTRODUCTION

Acute and chronic tendon disorders, most commonly affecting the calcaneus tendon (CT), account for up to 50% of all sports injuries (Jarvinen et al., 2005b; Egger and Berkowitz, 2017). Tendons are fundamental biomechanical structures that connect muscles to bone to produce movement through force transmission (Wang et al., 2012). Tendons improve the

economy of movement and amplify power output by their spring-like properties (Benjamin et al., 2008; Roberts and Azizi, 2010; Wilson and Lichtwark, 2011; Wang et al., 2012). Tendons also perform as mechanical buffers to protect skeletal muscle during contraction (Konow et al., 2012).

To perform this range of actions, tendons present abundant specialized extracellular matrix (ECM) resistant to tensile and compressive forces composed of approximately 70% of water by mass and collagen (mostly type I and III), proteoglycans, and other non-collagenous proteins organized in a hierarchical manner (Kjaer, 2004; Lavagnino et al., 2015). In addition, tendon contains a relatively small number of cells known as tenocytes arranged along collagen type I fibers forming an interconnected 3-dimensional network of cell and ECM. Importantly, the tenocytes transduce muscle-dependent loads to elicit functional remodeling of the ECM; tendons therefore demonstrate plasticity for dynamic adaptation in response to mechanical demands (Benjamin et al., 2008; Andarawis-Puri et al., 2015; Bohm et al., 2015; Lavagnino et al., 2015).

Homeostasis between tendon and muscle units requires a continuous bidirectional communication or cross-talk between tenocytes and muscle cells, known as myocytes (Schweitzer et al., 2010; Peng et al., 2017; Connizzo and Grodzinsky, 2018). Peng et al. (2017) reported that CT rupture and reconstruction led to "morphomechanical alterations" including a decrease of fascicle length, muscle thickness, and mechanical properties of the associated gastrocnemius muscle. This implies that tendon rupture and immobilization perturbed the bidirectional communication between tendon and muscle with functional consequences. On the other hand, Connizzo and Grodzinsky (2018) demonstrated that tendon can be affected by muscle using a mouse rotator cuff explant culture model which included the humeral head, supraspinatus tendon, and supraspinatus muscle. The authors found that pro-inflammatory cytokines from muscle and bone during stress deprivation culture, i.e., culture without mechanical stimuli, may cause tenocyte death after 3 days, which suggests a potential muscle-derived mechanism for onset of tendinopathy. Elsewhere, abnormal tendon loading such as underloading in cases of stress-shielding has been shown to induce an inflammatory response that weakens the structure and raise susceptibility to tendinopathies (Arnoczky et al., 2007; Cook and Purdam, 2009; Freedman et al., 2015; Bittencourt et al., 2016; Fouda et al., 2017). Such heterotypic interactions between tendon and skeletal muscle units may thus play a role in the etiology of tendinopathies and be relevant for clinical rehabilitation (Subramanian and Schilling, 2015).

Tendon morphogenesis is mediated by interactions with skeletal muscle *via* growth factors, protein migration, and force transmission (Freedman et al., 2015; Subramanian and Schilling, 2015). One of the major mechanosensitive pathways that control tendon responses to muscle-dependent loads during development and throughout life involves activation of latent transforming growth factor beta (*TGF-β*) in tendon ECM. This induces *SMAD* 2/3-dependent signal transduction and expression of transcription factors [e.g., mohawk homeobox (*MKX*), scleraxis (*SCX*), and *ERG1*] to induce collagen and proteoglycan synthesis (Subramanian and Schilling, 2015). Presumably, disruptions in muscle integrity as in the case of injury might lead to imbalances in these well-coordinated dynamic systems that in turn disrupt tendon integrity as well.

Although the cross-talk between tendon and muscle has been studied in recent years, to the best of our knowledge tendon plasticity in response to skeletal muscle injury has been neglected entirely. Thus, the aim of this study was to investigate the temporal effects of gastrocnemius muscle injury on the CT. We hypothesized that skeletal muscle injury would induce the CT to undergo maladaptive remodeling at the transcriptional level with concomitant harmful effects to morphology and biomechanical properties.

MATERIALS AND METHODS

Animals and Experimental Groups

All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (U.S. National Research Council, Washington D.C., USA). The research protocol received approval from the Animal Research Ethics Committee of the Catholic University of Brasilia, Brasília, Brazil (protocol number: 028/2015). Sixty-eight male Wistar rats (*Rattus norvegicus albinus*; weighing 300 +/− 25 g and 4 months of age at the start of experiments) were randomly divided into four experimental groups: control (C) group (animals not exposed to muscle injury) and 3, 14, and 28 days post gastrocnemius muscle (GA) injury (3D, 14D, and 28D). These time points were chosen to capture possible alterations in tendon during the three phases of the muscle regeneration process. Three days post-injure represents a degeneration/inflammation phase characterized by rupture and necrosis of the myofibers, formation of a hematoma, and inflammatory response. Fourteen days post-injure represents the remodeling phase characterized by maturation of regenerated myofibers with recovery of muscle function, fibrosis, and scar tissue formation. Twenty-eight days post-injure represents the complete or partial muscle maturation/functional repair. (Laumonier and Menetrey, 2016). The rats were placed in collective cages with members of the same group (3–4 animals/cage) with water and standard rodent chow (Purina®, Descalvado, São Paulo, Brazil) available *ad libitum* and exposed to 12 h light/dark cycles with temperature maintained at 20–22°C during the experiments.

Muscle Injury (Cryolesion) Protocol

The rats were anesthetized with an intraperitoneal injection of xylazine (12 mg/kg of body weight) and ketamine (95 mg/kg of body weight) for the surgical procedures. To induce muscle injury in the medial and lateral belly of the GA, the skin around the muscle was first trichotomized and cleaned. A transversal skin incision (1 cm) over the muscle belly was then performed to expose the GA muscle. To induce cryo-lesions, a rectangular iron bar (length = 4 cm; width = 0.4 cm

and height = 0.4 cm; total area = 6.56 cm^2) was frozen in liquid nitrogen and then applied twice for 10 s to the GA muscle belly. The same procedure was repeated twice with a 30 s interval in between. After that, the skin was sutured using nylon thread 4–0 (Shalon Medical). This protocol is a common procedure and has already used in other studies (Miyabara et al., 2006; Oliveira et al., 2006; Durigan et al., 2008; Vieira Ramos et al., 2016) promoting muscle injury in TA muscle. This model induces a homogeneous injury in GA restricted to the surface region of the muscle belly which produces injuries similar to muscle contusion lesions (Jarvinen et al., 2005a).

Muscle and Tendon Sample Collection

One at a time, the animals were euthanized using an intraperitoneal injection of xylazine solution (24 mg/kg of body weight) and ketamine (100 mg/kg of body weight) after the experimental periods and harvested. The CT was immediately dissected from posterior paws (3–8 min) and either; (1) frozen in RNase-free microtubes using liquid nitrogen (for qPCR) and then stored at −80°C; (2) fixed with 4% formaldehyde in (PBS, pH 7.4) for histological analysis; or (3) placed in physiological saline to prevent drying for mechanical testing. CT samples were approximately 8 mm in length and 1.5–2.5 mm in diameter.

Muscle Histological Analysis

Serial histological cross-sections of the GA belly muscles were obtained (10 μm) using a cryostat microtome (Microm HE 505, Jena, Germany). The cross-sections were stained with hematoxylin and eosin (HE, Sigma Aldrich, St. Louis, MO, USA). Pictures of the cross-sections were acquired using an Olympus BV51 microscope equipped with an SV Micro Sound Vision digital camera (Preston South, Australia) at 10× magnification.

To confirm the presence of GA muscle injury in the cryo-lesion model, the signs characterized were: the presence of tissue infiltration with polymorphonuclear cells, disrupted and hypercontracted muscle fibers as well as centralized nuclei as previously reported (Oliveira et al., 2006; Durigan et al., 2008; Vieira Ramos et al., 2016). Posteriorly, one histological cross-section of each GA muscle located in the central region of muscle injury was choose to measure the total cross-sectional area, injury area, and uninjured area of the muscle using a software for morphometry (Image J 1.44p - National Institutes of Health, Bethesda, MD). To measure those areas, previously, pictures of the cross-section of each muscle were obtained by light microscopy to reconstruct the total muscle cross-section using a software PTGui Pro version 11.15. These procedures permitted us to identify and measure the injury and uninjured areas of the muscle for clarifying the injury × recovery time points.

Calcaneus Tendon Histological Analysis

To evaluate morphological properties, the CT from 16 animals (4 per group) was fixed using 4% paraformaldehyde in phosphate-buffered saline (PBS) for 24 h at 4°C. After this step, they were washed in distilled water, serially dehydrated in ethanol, and embedded in paraffin.

Serial longitudinal sections (5 μm) of the peritendinous sheath and tendon proper were taken from the proximal and distal regions. The whole tendon was dissected out, and the longitudinal sectioning was cut (including proximal and distal regions) using glass knives and stained with hematoxylin and eosin (HE). This allowed investigation of adipose cells, blood vessels, and tendon cells from each region of the CT. Moreover, Masson's trichrome staining was performed according to the standard procedures to examine the general appearance of collagen deposition (Woo et al., 2006; Tang et al., 2014). Pictures of the longitudinal-sections were acquired as described above. The proximal region of CT was determined by the myotendinous junction to its middle portion, and the distal region was determined by the middle portion to the CT enthesis.

Image Analysis

For tendon H&E staining, 10 nonconsecutive digital images per region (peritendinous sheath and tendon proper, proximal and distal) were analyzed using Photoshop (Adobe Systems Inc., San Jose, CA, USA). A planimetry system using a translucent Weibel grid (Weibel, 1979) superimposed to each image was used to determine the volume density (Vv%) of adipose cells, blood vessels (lumen of blood vessel, endothelial cells, and perivascular sheath), other peritendinous sheath cells (includes all types of sheath cells, such as tenocytes, synovial cells, except adipose, and blood vessels cells), and tendon proper cells (fibroblasts and fibrochondrocyte-like cells). The stereology was performed by the point-counting method using coincidence of the grid points with the structures mentioned above. For the volume density estimation, the percentage of each structure in the peritendinous sheath and tendon proper was determined by multiplying the total number of grid points that coincide with the structures of interest by 100 and dividing by the total number of grid points falling on the peritendinous sheath or on the tendon proper.

ImageJ (National Institutes of Health, Bethesda, MD) was used in quantitative Masson's trichrome analysis (Hernandez-Morera et al., 2016). Collagen fibers were quantified using these steps: image acquisition and processing, setting the scale, deconvolution of the color images, and quantification of the collagen fibers. The entire protocol was performed as described previously by Chen et al. (2018).

RNA Extraction From TC Samples

Samples from 24 animals (6 per group) were homogenized in a tube containing five stainless steel balls (diameter, 2.3 mm) (BioSpec Products, Bartlesville, OK, USA) and three silicon-carbide sharp particles (1 mm) (BioSpec Products) by being shaken in a FastPrep-24 instrument (MP Biomedicals, Solon, OH, USA). To attain complete tissue homogenization, the shaking process was repeated seven times with ice cooling between each shaking step to help prevent RNA degradation. Total RNA

was extracted according to the TRIzol method described by Chomczynski and Sacchi (1987). A NanoDrop® spectrophotometer (ND-1000; NanoDrop Technologies Inc., Wilmington, DE, USA) was used to quantify RNA concentrations in each sample by determining the absorbance ratio of 260–280 nm. *TURBO DNA-freeKit* (Ambion – Life Technologies – 1907 M) was used for DNA digestion, according to the manufacturer's recommendations.

qRT-PCR Reverse Transcription

For the evaluation of CT gene expression, a total of 1 μg of RNA extracted from each tendon were converted into cDNA (final volume 20 μL) using *SuperScript™ VILO™ MasterMix* reverse transcriptase (Invitrogen-Cat. 11755-010) according to the manufacturer's protocol. To perform reverse transcription, the samples were incubated at 25°C for 10 min, at 42°C for 60 min, and at 85°C for 5 min before being stored at −20°C in a freezer.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

qRT-PCR was performed using *TaqMan Universal PCR Master Mix* system (Applied Biosystems, CA, USA – Cat. 4304437). Ten microliters of GoTaq Probe qPCR Master Mix (Promega – A6102) were homogenized and combined with 1 μL of the primer 20×, an amount of cDNA determined according to the standard curve, and water for a final volume of 20 μL. The amplification reaction was performed by QuantStudio™ 3 (Applied Biossystems) according to the manufacturer's instructions.

qRT-PCR was performed using a QuantStudio 3 Real-Time PCR System (Applied Biosystems) for the following genes: β-actin (*ACTB*), *ADAMTS-4*, biglycan (*BGN*), Type I collagen alpha 1 chain (*COL1A1*), Type III collagen alpha 1 chain (*COL3A1*), connective tissue growth factor (*CTGF*), decorin (*DCN*), *ERG1*, fibromodulin (*FMOD*), fibronectin (*FN*), glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), pro-insulin like growth factor IA (*IGF-1a*), interleukin-6 (*IL-6*), mohawk (*MKX*), matrix metalloproteinase-2 (*MMP-2*), ribosomal protein lateral stalk subunit P0 (*RPLP0*), scleraxis (*SCX*), Smad2 (*SMAD-2*), Smad3 (*SMAD-3*), syndecan-4 (*SDC-4*), transforming growth factor beta 1 (*TGF-β1*), tissue inhibitor of matrix metalloproteinase-1 (*TIMP-1*), tissue inhibitor of matrix metalloproteinase-2 (*TIMP-2*), tenascin C (*TNC*), tenomodulin (*TNMD*), and vascular endothelial growth factor (*VEGF*) (**Table 1**).

For each gene, all samples were amplified simultaneously with technical duplicates from the same cDNA in a single run. The expression of each target gene was normalized based on the expression of the constitutive RPLPO gene, which was used as the control of endogenous RNA, due to lower intra and intergroup variability compared to the other housekeeping genes tested (*GAPDH* and β-actin). The ΔCt values of the samples were determined by subtracting the mean Ct value of the target gene from the mean Ct value of the housekeeping gene. Subsequently, the ΔΔCt values were calculated by subtracting the ΔCt value of the condition of interest from the ΔCt of the control condition. Finally, $2^{-\Delta\Delta Ct}$ values were computed for presentation of relative expression data.

TABLE 1 | List of tested genes.

mRNA	Code (life technologies)	mRNA	Code (life technologies)
ACTB	rn00667869	*MKX*	rn01755203
ADAMTS-4	rn02103282	*MMP-2*	rn01538170
BGN	rn01529736	*RPLP0*	rn03302271
COL1A1	rn01463848	*SCX*	rn01504576
COL3A1	rn01437681	*SMAD-2*	rn00569900
CTGF	rn01537279	*SMAD-3*	rn00565331
DCN	rn01503161	*SDC-4*	rn00561900
ERG1	rn00561138	*TGF-β1*	rn00572010
FMOD	rn00589918	*TIMP-1*	rn01430873
FN	rn00569575	*TIMP-2*	rn00573232
GAPDH	rn01775763	*TNC*	rn01454947
IGF-1a	rn00710306	*TNMD*	rn00574164
IL-6	rn01410330	*VEGF*	rn01511602

β-actin (ACTB), ADAMTS-4, biglycan (BGN), Type I collagen alpha 1 chain (COL1A1), Type III collagen alpha 1 chain (COL3A1), connective tissue growth factor (CTGF), decorin (DCN), ERG1, fibromodulin (FMOD), fibronectin (FN), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), pro-insulin like growth factor IA (IGF-1a), interleukin-6 (IL-6), mohawk (MKX), matrix metalloproteinase-2 (MMP-2), ribosomal protein lateral stalk subunit P0 (RPLP0), scleraxis (SCX), Smad2 (SMAD-2), Smad3 (SMAD-3), syndecan-4 (SDC-4), transforming growth factor beta 1 (TGF-β1), tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), tissue inhibitor of matrix metalloproteinase-2 (TIMP-2), tenascin C (TNC), tenomodulin (TNMD), and vascular endothelial growth factor (VEGF).

It is important to highlight that the amount of sample and the efficiency of the reaction of each gene analyzed in the present study were determined from a standardization curve, having slope reference parameters equal to −3.3, R^2 = 0.9–1.0 and efficiency above 90%. All data generated or analyzed in this study were displayed at a database repository Gene Expression Omnibus (GEO, NCBI) (Accession-GSE131884)[1].

Biomechanical Tests

The test length and cross-sectional area (CSA) of tendons were determined by measuring the width and thickness of three different parts of the test region using digital calipers (Digimess Instrumentos de Precisão Ltda., São Paulo, SP, Brazil) and multiplying their means before the test. The 28 tendons (7 per group) were fixed to an Instron 5500R test instrument by clamps attached to their proximal and distal regions. Custom-sized clamps wrapped with filter paper were used to protect the specimens from macroscopic damage and/or slipping off during testing (Probst et al., 2000). Each tendon was stretched to failure at a constant displacement rate of 1 mm/min using a 0.2 kN load cell (S2 transducer force – S2M/200N-1 – HBM, Inc., USA) (Tohyama and Yasuda, 2000). The data were used to construct force-displacement curves from which the stress-strain curves were derived. Based on these curves, the following parameters were calculated: displacement at maximum load (mm),

[1]https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE131884

maximum load (N), maximum stress (MPa), maximum strain (%), elastic modulus (MPa), energy to failure (N-mm), and CSA (mm^2) according to de Cassia Marqueti et al. (2017) and Nakagaki et al. (2007). Each sample elastic modulus was calculated based on the slope of the linear portion of the stress-strain curve. All biomechanical tests for each time point were performed in a single day by a single operator.

Statistical Analysis

The results from gene expression were presented according to Heinemeier et al. (2007b) and Marqueti et al. (2018). The Shapiro-Wilk test was applied to check for normality of variables, and Levene's test was used to analyze homogeneity of variance. For the variables that did not have homogeneity of variance, the Welch test was applied to adjust the degrees of freedom of the residuals. Mean values were compared between groups by one-way ANOVA with the Bonferroni correction. In relation to the data that did not present normal distribution, the nonparametric Kruskal-Wallis test was applied. When appropriate pairwise comparisons were performed using Dunn's procedure with Bonferroni correction for multiple comparisons, the level of significance was $p \leq 0.05$. All analyses were conducted with statistical package for social sciences (SPSS, Inc., v. 21.0; IBM Corporation, Armonk, NY, USA) and GraphPad Prism 6.0 software (San Diego, California, USA). Stress-strain and force-displacement curves were generated using MATLAB (Release 2018A).

RESULTS

Morphology of Muscle Lesions

In order to evaluate the presence of muscle injury after cryo-lesion, qualitative analysis of histological sections stained with HE was performed in all experimental groups (**Figure 1**). Cross-sectional area of GA muscle in C group showed intact muscle fibers, with no sign of injury (**Figure 1A**). In contrast, in 3D group post-injury, necrotic fibers, hypercontracted fibers, and inflammatory infiltrate, labeled in the figure by the flags [hash (#), paragraph (§), and asterisk (*), respectively], indicated the lesion was in the inflammatory phase of healing (**Figure 1B**). In the 14D group post-injury, centralized nuclei were visible (indicated in the figure by the black arrow), characterizing a regenerative process (**Figure 1C**). Finally, in the 28D group post-injury central nucleation had almost disappeared (a sign of regeneration in injured muscles) (Vieira Ramos et al., 2016), evidencing that muscle regeneration was nearing completion. In general, the characteristics described above confirm that cryo-lesion caused injury in the GA muscle.

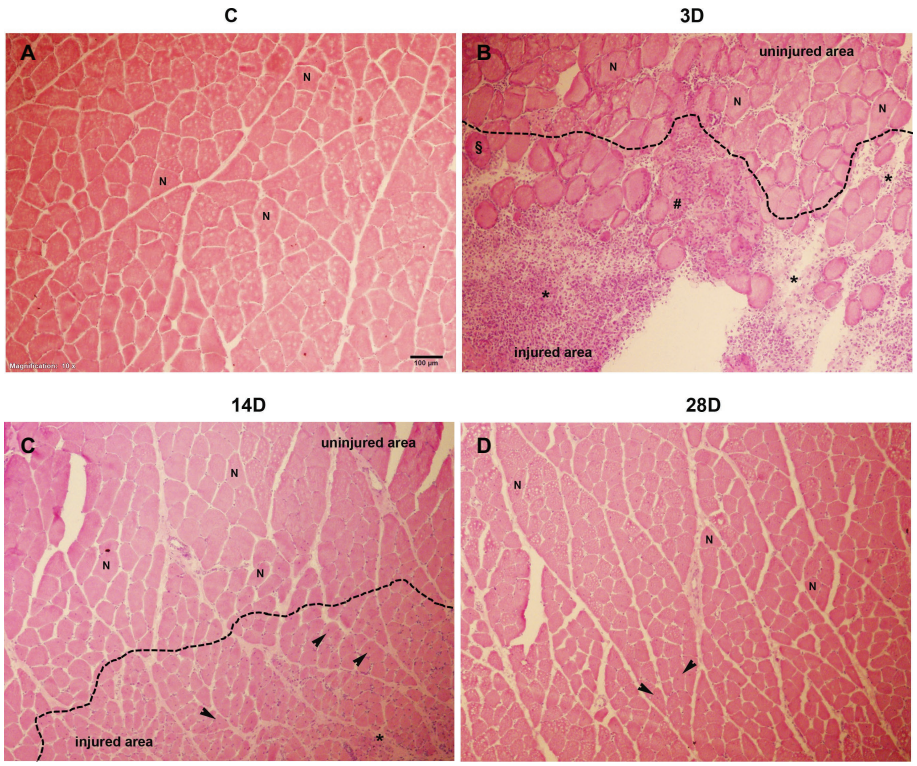

FIGURE 1 | Histological sections of the muscle healing process of gastrocnemius muscle in four groups post-operation hematoxylin-eosin (HE) staining: **(A)**, control (C); **(B)**, 3 days post gastrocnemius muscle injury (3D); **(C)**, 14 days post gastrocnemius muscle injury (14D); **(D)**, 28 days post gastrocnemius muscle injury (28D). Inflammatory infiltrate (*), necrotic fiber (#), hypercontrated fibers (§); centralized nucleus (black arrow), and fibers with normal appearance (N). Magnification: 10×. The bar represents 100 μm.

TABLE 2 | Total and uninjured cross-sectional area of gastrocnemius muscle.

Groups	Animal weight (g)	Muscle weight (g)	Total area (mm^2)	Injury area (mm^2)	Injury area (%)
C	340.0 ± 23.85	1.66 ± 0.16	15.975 ± 1.542	0	0
3D	354.5 ± 34.6	1.67 ± 0.15	14.678 ± 2.303	1.773 ± 330.5[a]	12.1
14D	338.8 ± 22.2	1.56 ± 0.05	10.374 ± 1.141[a,b]	0.581 ± 0.13[a,b]	5.6
28D	361.5 ± 38.0	1.63 ± 0.15	12.484 ± 921.4[a]	0[b,c]	0

The data are mean ± SD. The experimental groups: control (C), 3 days (3D), 14 days (14D), and 28 days post gastrocnemius muscle injury (28D).
[a]*Significantly different from C.*
[b]*Significantly different from 3D.*
[c]*Significantly different from 14D, $p < 0.05$. (n = 4 per group).*

Animals Weight, Muscle Weight, and Injury and Uninjured Cross-Sectional Area of GA Muscle Middle Belly

We observed higher injury area in the 3D and 14 D groups when compared with the C group, respectively ($p = 0.001$; $p = 0.001$), besides the 14D group showed the lowest values when compared to the 3D group ($p = 0.001$). Additionally, the 28D group displayed lower injury area when compared with the 3D and 14D group, respectively ($p = 0.001$; $p = 0.001$). The total area was lower in the 14D group when compared with the C and 3D group, respectively ($p = 0.001$; $p = 0.01$; **Table 2**). No changes were observed in animals and muscle weight among the experimental groups ($p > 0.05$).

Muscle Injury Alters Gene Expression in Rat Calcaneus Tendon

In order to analyze the GA lesion impact on CT, the expression of key genes that regulate the ECM in tendon homeostasis and remodeling was evaluated. Gene expression analysis was normalized by constitutive gene *RPLP0*. We also tested housekeeping genes *β-actin* and *GAPDH* but they were not stable; notably, *GAPDH* was the least stable gene expressed of all genes tested.

Growth factors: *TGF-β1* mRNA levels were upregulated in the 28D group when compared with C and 3D groups, respectively ($p = 0.02$; $p = 0.001$; **Figure 2A**). No changes were observed in mRNA levels of *VEGF* gene expression among the groups ($p > 0.05$; **Figure 2B**). *IGF-1a* mRNA levels were significantly upregulated in the 28D group when compared with the 3D group ($p = 0.02$; **Figure 2C**). *CTGF* mRNA levels were downregulated in the 3D group when compared with the C group ($p = 0.001$; **Figure 2D**), whereas the 14D and 28D groups demonstrated upregulation when compared with the 3D group ($p = 0.02$ and $p = 0.01$, respectively; **Figure 2D**).

Structural proteins: *COL1A1* mRNA levels were downregulated in the 3D group when compared with the C group ($p = 0.03$; **Figure 2E**). However, the 14D group showed upregulation when compared with the 3D group ($p = 0.001$; **Figure 2E**). Regarding *COL3A1*, the 14D group displayed upregulation in mRNA levels when compared with the C group ($p = 0.02$; **Figure 2F**).

Small leucine rich proteoglycans (SLRP): *DCN* mRNA levels were significantly upregulated in the 28D group when compared with the 3D group ($p = 0.001$; **Figure 2H**). No changes were observed in mRNA levels of *BGN* and *FMOD* ($p > 0.05$; **Figures 2G,I**).

Matrix remodeling enzymes: *MMP-2* mRNA levels were upregulated in the 3D group when compared with the C group ($p = 0.02$; **Figure 2J**), whereas the 14D and 28D groups demonstrated upregulation when compared with the 3D group, respectively ($p = 0.001$; $p = 0.001$; **Figure 2J**). Additionally, *TIMP-1* mRNA levels were downregulated in the 28D group when compared with the C and 3D group, respectively ($p = 0.001$; $p = 0.002$; **Figure 2K**). On the other hand, *TIMP-2* mRNA levels were downregulated in the 3D group when compared with the C group ($p = 0.01$; **Figure 2L**); however, the 14D and 28D groups showed upregulation compared with the C ($p = 0.01$; $p = 0.04$ **Figure 2L**) and 3D groups, respectively ($p = 0.001$; $p = 0.001$; **Figure 2L**).

Cytokine: No changes were observed in mRNA levels of IL-6 among the groups ($p > 0.05$; **Figure 2M**).

Developmental/progenitor and differentiation factors: *TNMD* mRNA levels were significantly upregulated in the 28D group when compared with the C, 3D, and 14D groups, respectively ($p = 0.01$; $p = 0.001$; $p = 0.001$; **Figure 2N**). No changes were observed in mRNA levels of *SMAD-2* among the groups ($p > 0.05$; **Figure 2O**). However, *SMAD-3* mRNA levels were upregulated in the14D group when compared with the 3D group ($p = 0.01$; **Figure 2P**), but the 28D displayed downregulation when compared with the 14D group ($p = 0.01$; **Figure 2P**). *SCX* and *ERG-1* mRNA levels were significantly upregulated in the 28D group when compared with the C ($p = 0.001$; $p = 0.02$; **Figures 2Q,T**), 3D (p = 0.002; p = 0.001; **Figures 2Q,T**), and 14D groups, respectively (p = 0.001; p = 0.004; **Figures 2Q,T**). Moreover, *MKX* mRNA levels were upregulated in CT of the 14D and 28D groups when compared with the C ($p = 0.04$; $p = 0.001$; **Figure 2R**) and 3D groups, respectively ($p = 0.001$; $p = 0.001$; **Figure 2R**). No changes were observed in mRNA levels of *ADAMTS-4* among the groups ($p > 0.05$; **Figure 2S**).

Matricellular proteins: No changes were observed in mRNA levels of *SDC-4* among the experimental groups ($p > 0.05$). However, FN and TNC mRNA levels were significantly upregulated in the 14D and 28D groups when compared with the 3D group, respectively ($p = 0.01$; $p = 0.01$; $p = 0.01$; $p = 0.01$; **Figures 2G,H,U,V,X**).

Light Microscopy of Tendon Hematoxylin and Eosin Staining

HE staining was performed to investigate adipose cells, blood vessels, peritendinous sheath cells, and tendon proper cells in

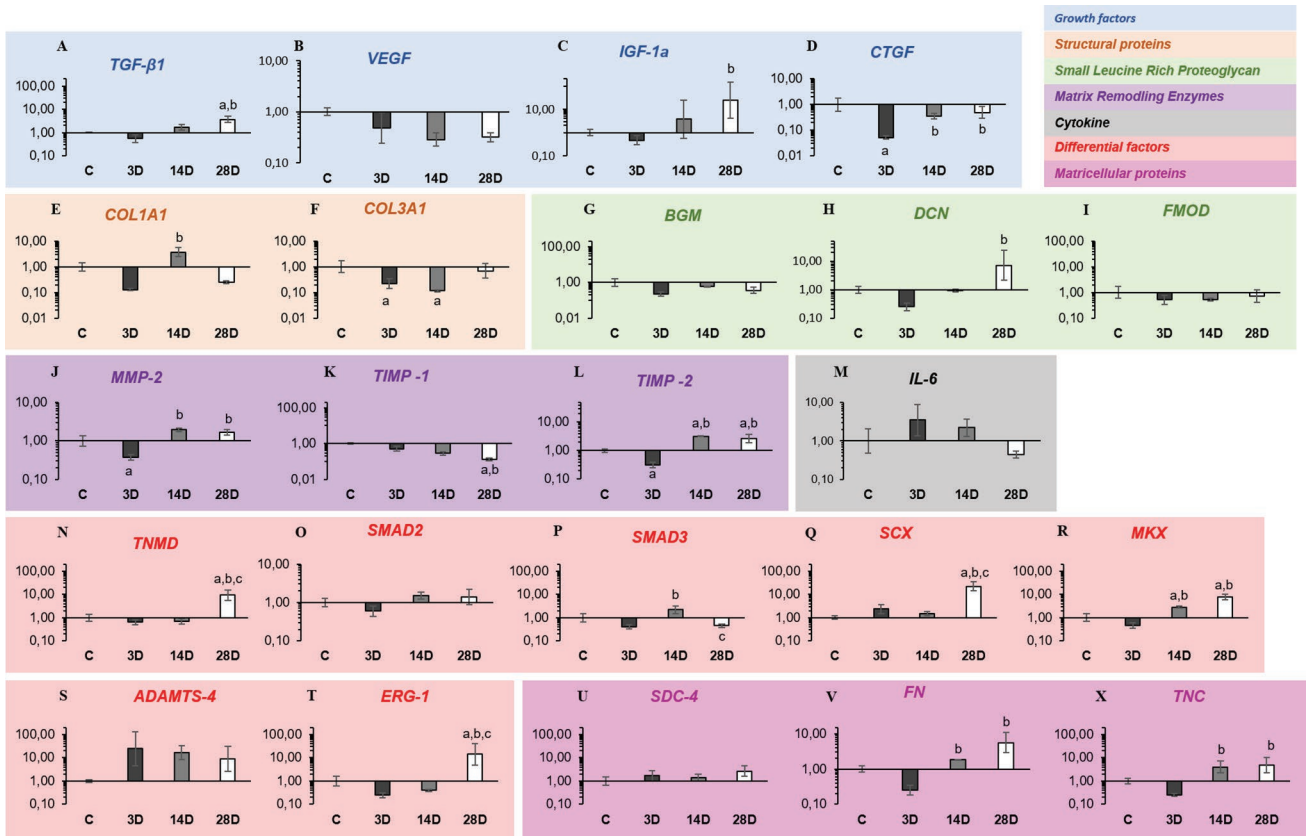

FIGURE 2 | Gastrocnemius muscle injury modifies the gene expression related to ECM synthesis, remodeling, and tendon repair. **(A–D)** Growth factors (blue): transforming growth factor-beta 1 (*TGF-β1*), vascular endothelial growth factor (*VEGF*), pro-insulin like growth factor IA (*IGF-Ia*), and connective tissue growth factor (*CTGF*). **(E,F)** Structural proteins (light orange): collagen, type I, alpha 1 (*COL1A1*) and collagen, type III, alpha 1 (*COL3A1*). **(G,I)** Small leucine-rich proteoglycan (green): biglycan (*BGN*), decorin (*DCN*), and fibromodulin (*FMOD*). **(J–L)** Matrix remodeling enzymes (violet): matrix metalloproteinase-2 (*MMP-2*), tissue inhibitor of matrix metalloproteinases-1 (*TIMP-1*), and tissue inhibitor of matrix metalloproteinases-2 (*TIMP-2*). **(M)** Cytokine (gray): interleukin-6 (*IL-6*). **(N–T)** Differential factors (beige): tenomodulin (*TNMD*), Smad2 (*SMAD2*), Smad3 (*SMAD3*), scleraxis (*SCX*), mohawk (*MKX*), *ADAMTS-4*, and *ERG-1*. **(U–X)** Matricellular proteins (pink): Syndecan-4 (*SDC-4*), fibronectin (*FN*), and tenascin C (*TNC*). The experimental groups: control (C), 3 days post gastrocnemius muscle injury (3D), 14 days post gastrocnemius muscle injury (14D), 28 days post gastrocnemius muscle injury (28D). a, significantly different from C; b, significantly different from 3D; c, significantly different from 14D. $p < 0.05$ (n = 6 per group).

proximal and distal regions of CT (**Figures 3A-P,A1-J1**). The 14D group presented lower adipose cell Vv% in the proximal region of the CT when compared with 3D group (p = 0.01; **Figures 3K,A1**). Moreover, the 28D group displayed decreased adipose cell Vv% when compared with the C and 3D groups, respectively (p = 0.001; p = 0.001; **Figures 3O,B1**). We also observed higher blood vessel Vv% in the proximal region of tendon proper in the 14D group when compared with the C group (p = 0.002; **Figures 3I,E1**); however, the 28D group showed lower blood vessel Vv% in the same region of tendon proper when compared with the 14D group (p = 0.01; **Figures 3M,E1**). Finally, 14D and 28D groups demonstrated increased peritendinous sheath cell Vv% in the proximal region when compared with the C (p = 0.04; p = 0.001; **Figures 3K,O,G1**) and 3D groups, respectively (p = 0.04; p = 0.001; **Figures 3K,O,G1**).

No significant differences were observed between the groups with respect to blood vessel Vv% of peritendinous sheath and tendon proper cell Vv% in the proximal region of the CT ($p > 0.05$; **Figures 3C,I1**).

On the other hand, the 28D group presented lower cell Vv% in the distal region of tendon proper of the CT when compared with the C and 14D groups, respectively (p = 0.04; p = 0.04; **Figures 3N,J1**). Lastly, no significant differences were observed between groups with respect to adipose cell Vv% and blood vessel Vv% of peritendinous sheath and in tendon proper and peritendinous sheath cell Vv% in the distal region of the CT ($p > 0.05$; **Figures 3B1,D1,F1,H1**).

Light Microscopy of Tendon Masson's Trichrome Staining

Subsequently, the collagen content was analyzed in CT by Masson's trichrome staining. Collagen content decreased in the distal region of the 3D group when compared with the C group (p = 0.02; **Figures 4C,I**). The 14D group displayed lower collagen content when compared with the C and 3D groups, respectively (p = 0.001; p = 0.03; **Figures 4E,I**). However, collagen content increased in the 28D group when compared with the 3D and 14D groups, respectively (p = 0.01; p = 0.001; **Figures 4G,I**).

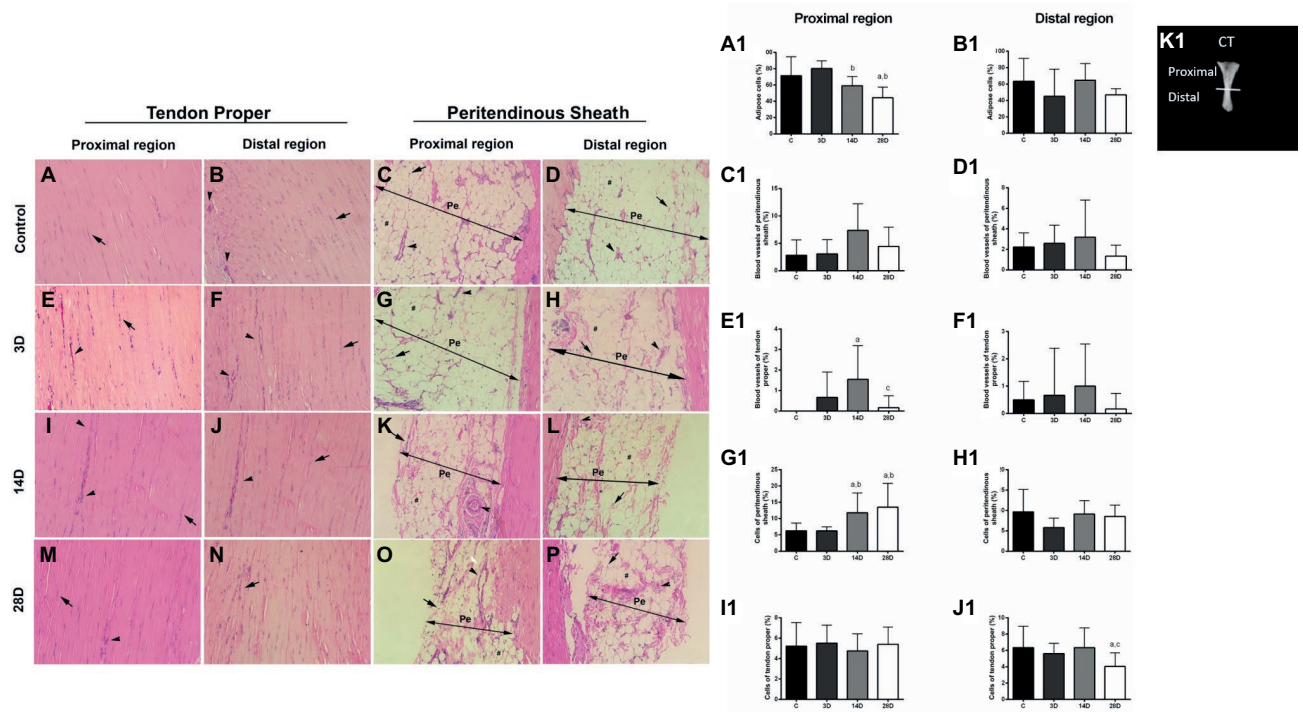

FIGURE 3 | Morphological properties in calcaneal tendon are altered following gastrocnemius muscle injury. **(A1–J1)** express Vv% variation of structural elements found in the proximal and distal regions of the CT in each experimental group. **(A–P)** evidenced longitudinal sections (5 μm) of the proximal and distal regions of CT [tendon proper and peritendinous sheath (Pe) stained with hematoxylin-eosin]. Control group (C): tendon proper of the proximal region **(A)** and distal region **(B)** indicating cells (compact arrow) and vessels (arrowhead). Pe of proximal region **(C)** and distal **(D)** indicating cells (compact arrow), adipose cells (asterisk), and vessels (arrowhead); 3 days post gastrocnemius muscle injury (3D): tendon proper of the proximal region **(E)** and distal region **(F)** indicating cells (compact arrow) and vessels (arrowhead). Pe of the proximal **(G)** and distal **(H)** regions indicating cells (compact arrow), adipose (asterisks), and vessels (arrowhead); 14 days post gastrocnemius muscle injury (14D): tendon proper of the proximal region **(I)** and distal region **(J)** indicating cells (compact arrow) and vessels (arrowhead). Pe of the proximal **(K)** and distal **(L)** regions indicating cells (compact arrow), adipose (asterisks), and vessels (arrowhead); 28 days post gastrocnemius muscle injury (28D): tendon proper of the proximal region **(M)** and distal region **(N)** indicating cells (compact arrow) and vessels (arrowhead). Pe of the proximal **(O)** and distal **(P)** regions indicating cells (compact arrow), adipose (asterisks), and vessels (arrowhead). **(K1)** shows the dissected CT (adapted from Covizi et al., 2001) and indicates the position corresponding to proximal and distal regions. The data are mean ± SD. a = significantly different from C; b = significantly different from 3D; c = significantly different from 14D; $p < 0.05$. Magnification: 400×. The bar represents 40 μm. ($n = 4$ per group).

With regard to the proximal region, collagen content decreased in the 14D group when compared with the C and 3D groups, respectively ($p = 0.001$; $p = 0.01$; **Figures 4F,J**), whereas collagen content increased in the 28D in comparison with 14D group ($p = 0.01$; **Figures 4H,J**).

Tendon Biomechanical Properties Analysis

Figure 5 shows the force-displacement curves (A) obtained for the C, 3D, 14D, and 28D groups, from which the stress-strain curves were derived (**Figure 5B**). Of the six samples in each group that were subject to tensile testing, any that showed a sudden drop in force without breaking, i.e., evidence of slipping, on the force-displacement curves were excluded from the analysis: 0/6 from C; 1/6 from 3D; 2/6 from 14D; 1/6 from 28D. These samples otherwise, showed similar force-displacement profiles to those in their respective experimental groups; thus, the slipping was likely due to insufficient clamping of the samples rather than those samples being of particularly high-stiffness. For this reason, we do not believe that excluding them introduced a selection bias into the study.

Differences in the profile of stress-strain curves (**Figure 5B**), the 28D showed a greater capacity of resistance to tensional load despite of stiffnesses observed when compared with others groups. The analysis of the material properties was based on these curves and will be described below.

The maximum load in CT increased in 28D when compared with the 3D group ($p = 0.02$; **Figure 6A**). Regarding tendon extension (displacement at maximum load), the 3D group showed a lower maximum extension when compared to the control group ($p = 0.04$). In addition, 28D group decreases tendon extension compared to the other experimental groups ($p = 0.02$; **Figure 6B**). The maximum stress in tendon increased in 28D when compared with the 3D group ($p = 0.02$; **Figure 6C**).

The 3D group showed a decrease of maximum strain compared to control group ($p = 0.001$). However, the 14D group showed an increase of maximum strain compared to C and 3D groups, respectively ($p = 0.001$ and $p = 0.001$). Additionally, 28D displayed lower maximum stress when compared to the other experimental groups ($p = 0.001$; **Figure 6D**). Finally, 3D showed increased elastic modulus

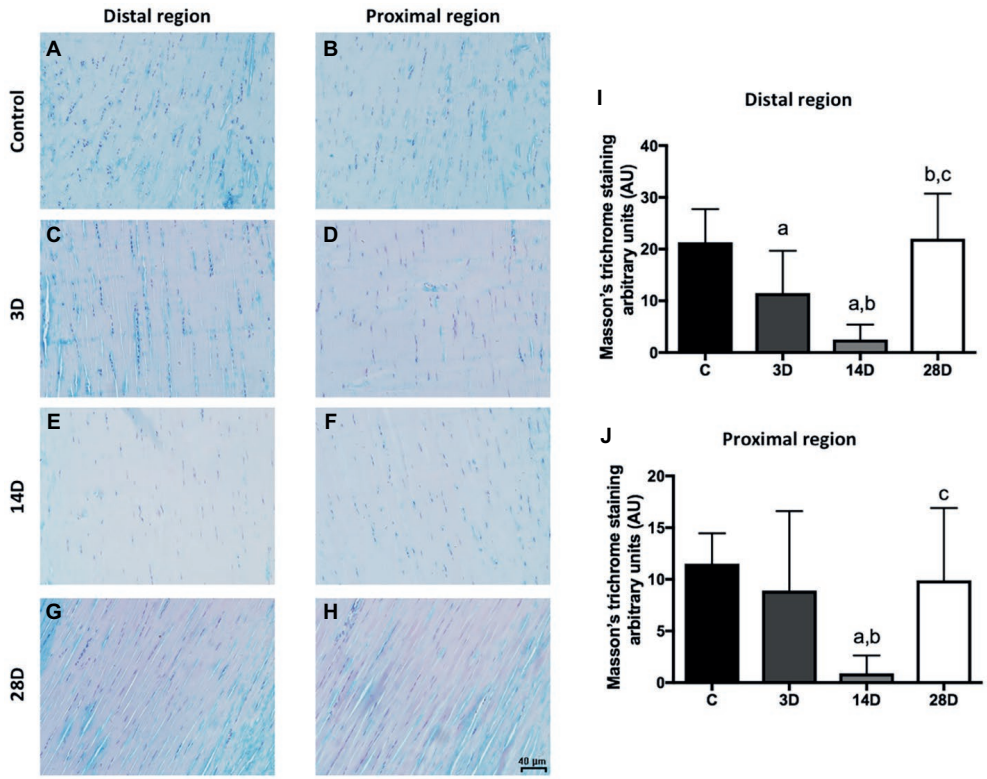

FIGURE 4 | Masson's trichrome staining indicated a lower collagen content of proximal and distal regions of the CT after gastrocnemius muscle injury. **(A–H)** evidenced longitudinal sections (5 µm) of the proximal and distal regions of CT (tendon proper). Collagen content (AU) in the proximal **(I)** and distal **(J)** regions. The data are mean ± SD. The experimental groups (n = 5): control (C), 3 days post gastrocnemius muscle injury (3D), 14 days post gastrocnemius muscle injury (14D), 28 days post gastrocnemius muscle injury (28D). a = significantly different from C; b = significantly different from 3D; c = significantly different from 14D; $p < 0.05$. Magnification: 400×. The bar represents 40 µm. (n = 4 per group).

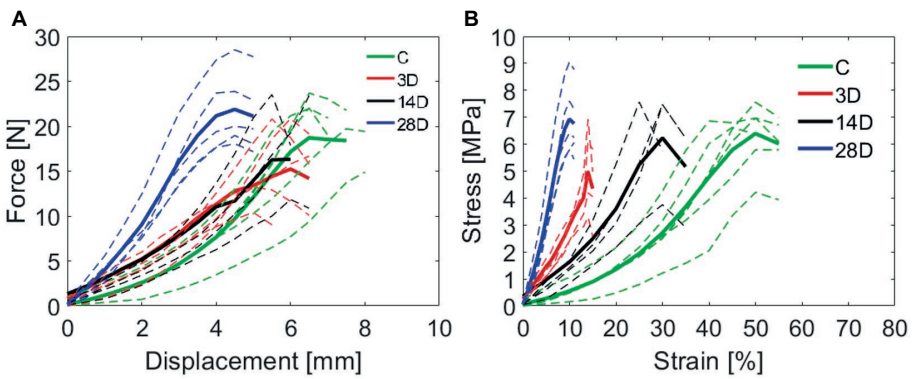

FIGURE 5 | (A) Gastrocnemius muscle injury affects calcaneal tendon force-displacement curves. The experimental groups: control (C), 3 days (3D), 14 days (14D), and 28 days post gastrocnemius muscle injury (28D). **(B)** Calcaneal tendon stress-strain curves obtained after gastrocnemius muscle injury. Dotted lines indicate all the tested samples into each group and the thick line indicate the mean of the group. (n = 7 per group).

when compared with the control group and 28D groups showed the highest elastic modulus values when compared to the other groups (p = 0.016 and p = 0.001; **Figure 6E**). No significant differences were observed between groups with respect to energy to failure and cross-sectional area ($p > 0.05$; **Figures 6F,G**).

DISCUSSION

This study investigated the effects of skeletal muscle injury on time-course effects on the expression of key genes involved in tendon remodeling as well as the morphological and biomechanical properties of the calcaneal tendon (CT).

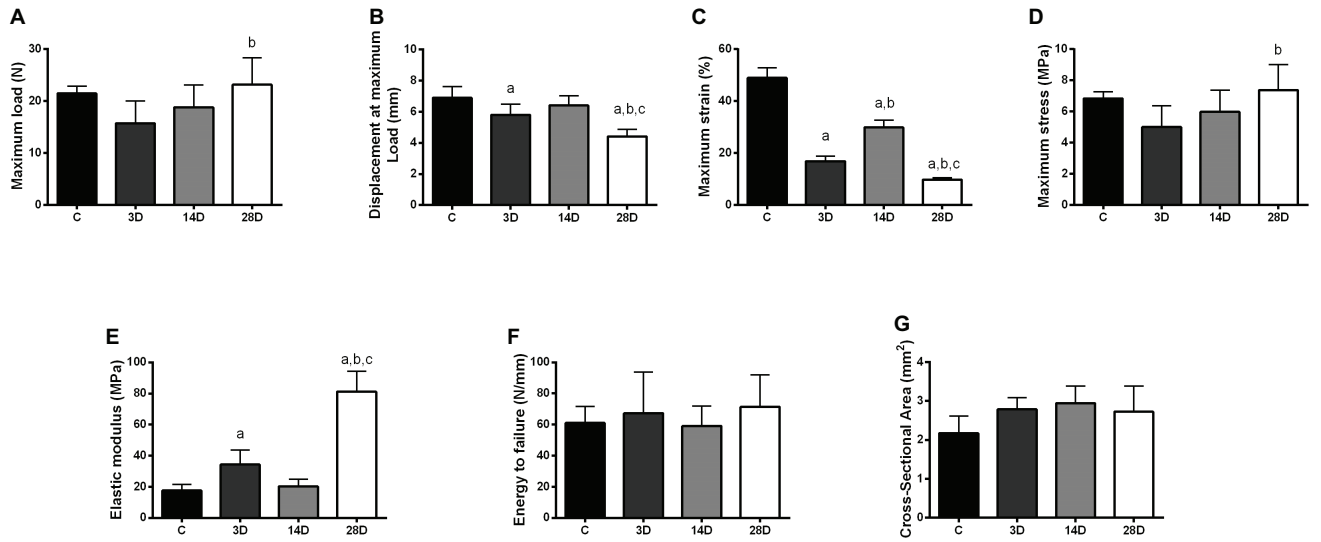

FIGURE 6 | Biomechanical properties modifications in the calcaneal tendon in response to gastrocnemius muscle injury. Maximum load **(A)**, displacement at maximum load **(B)**, maximum stress **(C)**, maximum strain **(D)**, elastic modulus **(E)**, energy to failure **(F)**, and cross-sectional area **(G)** presented. The data are mean ± SD. The experimental groups: control (C), 3 days (3D), 14 days (14D), and 28 days post gastrocnemius muscle injury (28D). a = significantly different from C; b = significantly different from 3D; c = significantly different from 14D, $p < 0.05$. ($n = 7$ per group).

For the first time, the present study demonstrated tendon plasticity in terms of gene expression as well as morphological properties and biomechanical adaptations in response to skeletal muscle injury. The morphological analysis was performed in the proximal and distal regions of CT. Although the proximal and distal regions are basely fibrous, the distal region of the CT is subjected to different levels of compressive forces and friction than the proximal region (Covizi et al., 2001). It was observed that distinct regions of CT differ in its response to muscle injury stimuli. Our new understanding of tendon plasticity in response to skeletal muscle injury may have crucial implications for treatment of tendinopathy.

Tendon Response at Three Days Post Muscle Injury (3D)

The muscle showed the greatest disruption at 3D, indicating that the CT might also have been affected at this time point (**Figure 1**). Modulation in *CTGF* and *COL1A1* gene expression has been previously reported after tendon perturbations such as exercise (net collagen degradation after 24–36 h followed by net synthesis by up to 72 h) (Magnusson et al., 2010) and tendinopathies models (reduction in *COL1A1* both 24 and 72 h of glutamate exposure) (Dean et al., 2015). The novelty of our study was that skeletal muscle injury also decreases *CTGF* and *COL1A1* gene expression in tendon after 3 days. These results coincide with decreased collagen content in the distal tendon region at the same time, indicating what may be a progression toward a degenerative status. *CTGF* modulates many biological processes in tendon, including cell adhesion, migration, proliferation, and angiogenesis (Chiquet et al., 2003; Nakama et al., 2006; Liu et al., 2015b). Blockade of *CTGF* action or inhibition of *CTGF* synthesis may be associated with a decrease of collagen synthesis in fibroblast cellular culture (Duncan et al., 1999), which can cause detrimental effects on structural support, protection, and maintenance in tendon functional integrity. A plausible explanation for the downregulation of *CTGF* and *COL1A1* and decreased collagen content at 3D could be the lack of mechanical stimulus in tendon induced by muscle injury (i.e., stress shielding), since previous studies reported that mechanical stress can induce a high-level of *CTGF* expression and collagen I synthesis (Schild and Trueb, 2002; Chiquet et al., 2003; Heinemeier et al., 2007a; Galloway et al., 2013). These data suggest that collagen homeostasis is disrupted at 3D following muscle injury.

The ECM homeostasis is in part controlled by the balance of *MMPs* and *TIMPs* (Minkwitz et al., 2017); thus, the decrease of *MMP-2* and *TIMP-2* at 3D post injury suggests that the tendon was undergoing remodeling in response to the skeletal muscle injury. In addition, muscle injury likely hinders the normal molecular interactions between tendon and skeletal muscle such as *MMPs*, growth factors, and migration/proliferation of proteins that modulate tendon morphogenesis (Subramanian and Schilling, 2015; Thankam et al., 2016). Overall, muscle injury appeared to disrupt normal homeostatic molecular signaling in CT.

The disruption at the gene expression level at 3D in CT was associated with a disruption in biomechanical properties. Specifically, the maximum strain in tendons was significantly reduced by 2.9-fold at 3D stress-strain curve; further, the stress-strain curve appeared considerably steeper compared to the uninjured control. This demonstrates that just 3D of muscle

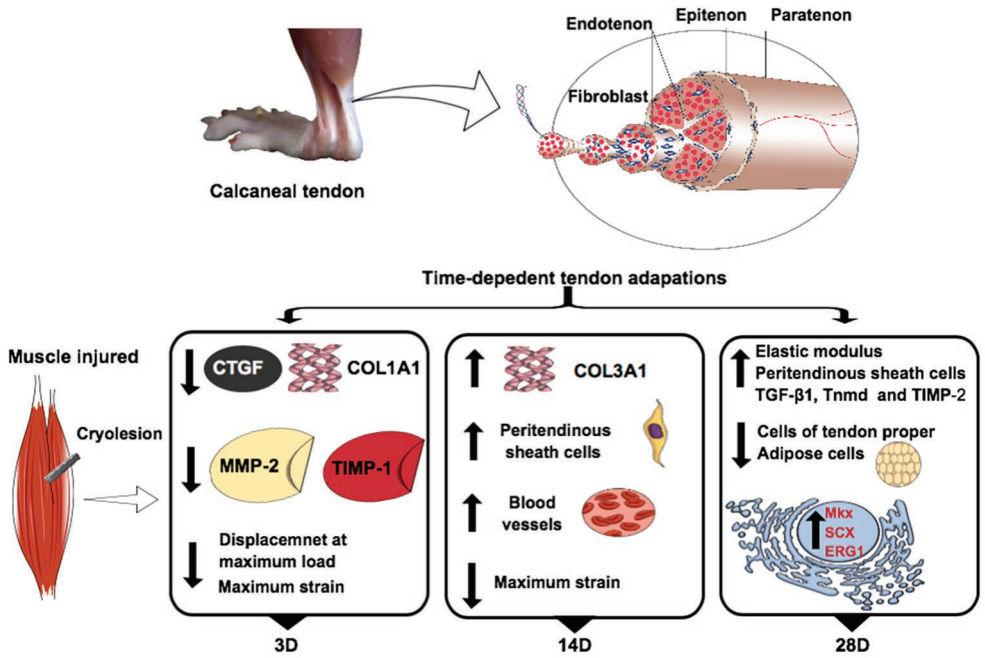

FIGURE 7 | Skeletal muscle injury modulates calcaneal tendon plasticity. Main temporal analyses of the gene expression, morphological, and biomechanical properties of calcaneal tendon in relation to the control group. The experimental groups: control (C), 3 days (3D), 14 days (14D), and 28 days (28D) post gastrocnemius muscle injury. Connective tissue growth factor (*CTGF*), collagen, type I, alpha 1 (*COL1A1*), collagen, type III, alpha 1 (*COL3A1*), matrix metalloproteinase-2 (MMP-2), tissue inhibitor of matrix metalloproteinases-1 (*TIMP-1*), tissue inhibitor of matrix metalloproteinases-2 (*TIMP-2*), tenomodulin (*TNMD*), mohawk (*MKX*), scleraxis (*SCX*), and *ERG1*. The tendon calcaneal figure was adapted from Guzzoni et al. (2018).

injury was sufficient to induce considerable mechanical alterations in tendon with reduced ability to withstand strain. Previous studies have shown that tendon biomechanical properties depend upon small leucine-rich proteoglycans (SLRPs; decorin and biglycan in particular) (Robinson et al., 2017). SLRPs are the predominant proteoglycans in tendon with decorin accounting for around 80% of the total proteoglycan content of the tissue (Samiric et al., 2004). In the present study, there were downward trends in gene expression of both decorin and biglycan at 3D relative to the control.

The tendon ECM remodeling and biological function are intimately linked to mechanical load and muscle contraction intensity (Galloway et al., 2013). It has been demonstrated that external controlled load through exercise is a primary factor to promote tissue remodeling, which can lead to structural and functional improvements (Galloway et al., 2013). However, overloading above the tendon capacity is widely studied for their potential damaging factor, as well as the abolition of muscle load (Millar et al., 2017). The muscle injury performed in the present study showed a great traumatic process in the muscle. Experimental studies have demonstrated biomechanical changes in CT only after 4 weeks of complete stress shielding in calcaneus tendon (Ikoma et al., 2013; Kaux et al., 2013). Likewise, at least 5 weeks of high-power strength training protocols, focused on the triceps sural complex, have been shown useful in modifying mechanical variables (Kubo et al., 2007). This factor demonstrates that mechanical privacy (by the abrupt decrease of muscle activity) in the current study have been enough to generate significant mechanical alterations in the tendon.

Fourteen Days Post Injury (14D)

Types I and III collagen showed variable expression over time. The upregulation of *COL1A1* post 14 days was associated with increased *MKX* and *SMAD3*, which are important regulators of ECM production including expression of *COL1A1* (Berthet et al., 2013; Liu et al., 2015a). In contrast, *COL3A1* showed a trend toward a decrease at 3D post-injury relative to the control followed by a further decrease at 14D. At the same time, Masson's trichrome staining revealed lower collagen content at 14D relative to the control and the 3D post injury group. The decline in collagen content at 14D may thus be reflected in the shallower stress-strain curve at 14D relative to 3D. We might speculate that the increase of *COL1A1* gene expression at 14D post-muscle injury may reflect a compensatory mechanism, producing collagen in response to the altered mechanical properties at 3D.

These findings with respect to type I collagen may be supported by the abundance of peritendinous sheath cells found in the 14D post skeletal muscle injury group. These cells may have roles in restoring collagen content. In addition, the upregulation of *CTGF, FN, MMP-2, TIMP-2, SMAD3*, and *MKX* 14 days post injury in comparison to 3D, indicating

that tendon undergoes adaptation following skeletal muscle injury. This may have clinical relevance in the prevention and treatment of musculoskeletal injuries (Benjamin et al., 2008; Bohm et al., 2015).

While blood vessels in the control group were limited to the peritendinous sheath, we found them trend toward an increase in abundance at 14D in the peritendinous sheath and penetrating the tendon proper. This may have provided the nutritional and metabolic support for the active ECM remodeling in the CT in response to skeletal muscle injury. However, we also observed a downward trend in *VEGF* gene expression in 3D and 14D compared to the control group. Further investigations are required to explain this contradictory result; it may suggest that other adjacent molecular pathways or post-translational regulation were involved in producing the observed vascularization.

Twenty-Eight Days Post Injury (28D)

Twenty-eight days post skeletal muscle injury had increased peritendinous sheath cell content in the proximal region of tendon compared with the control group. Moreover, 28D showed similar collagen content to the control group, suggesting recovery from the loss of collagen observed at 14D. These aspects may be linked to increased *TGF-β1* expression observed at 28D. *TGF-β1* is an important factor in stimulating ECM growth, turnover, and remodeling, being a well-known promoter of tendon fibroblast proliferation and collagen synthesis (Kjaer, 2004; Gumucio et al., 2015; Goodier et al., 2016). *SCX*, *MKX*, and *ERG1*, which are important transcription factors during tendon development and repair, also appear to regulate expression of matrix molecules as *TNMD* which is consistent with the concurrent upregulation of all these genes at 28D (Murchison et al., 2007; Shukunami et al., 2016; Wu et al., 2017). As well, these genes are regulated by the *TGF-β* superfamily (Mendias et al., 2012; Subramanian and Schilling, 2015).

SCX regulates fibroblast proliferation and initiation of tenocyte differentiation in response to mechanical stimuli (Murchison et al., 2007). Previously, decreased *SCX* was shown to be related to removal of tendon load, whereas it increased dramatically in response to physiological loading (Mendias et al., 2012). For example, Murchison et al., 2007 reported that mice with loss *SCX* expression have a disorganized ECM and reduced collagen which compromise the power transmission capacity of tendon. Thus, these adaptations post 28 days skeletal muscle injury may be important to maintain joint movement, passive elastic response, and the restoration of tendon strength (Subramanian and Schilling, 2015). For their part, *MKX* and *ERG1* were shown to be involved in the maintenance of differentiated tenocytes (Wu et al., 2017).

TNMD is a tendon-specific gene marker, known to be important for tendon maturation (Docheva et al., 2005). *TNMD* plays a role in proliferation, differentiation, and migration of tendon progenitor cells (TSPCs) as well as endothelial cells (Shukunami et al., 2005, 2016). Our findings demonstrate that regulation of *SCX, MKX, ERG1, TGF-β1*, and *TNMD* are linked to increased muscle activity. This agrees with previous studies showing overexpression of *TGF-β, SCX*, and *TNMD* induced by mechanical loading.

Previous studies have found structural (i.e., collagen fibrils irregularly arranged and loose) and biomechanical changes (i.e., poor dynamic storage energy) in CT after few weeks of complete stress shielding in calcaneus tendon (44, 45). In respect to biomechanical properties following skeletal muscle injury, it appears that after 28D, the CT retains similar load-bearing capacity to the uninjured control. However, the CT appeared to lose flexibility at 28D, given that it had increased Young's modulus compared to all time points and the least ability to withstand high levels of strain. The increase in Young's modulus at 28D seemed to correlate with collagen synthesis and collagen fiber organization in this period. In a future study, it may be interesting to see if these changes in biomechanical properties persist several months after the skeletal muscle injury, and whether these changes can be linked to future susceptibility to tendon injury.

Another novelty of our study was that 28 days post GA injury showed decreased adipose cells in tendon when compared with the control group. Previous data have already shown that high amounts of adipose cells may indicate tendon degeneration and deleterious effects of aging (Benjamin et al., 2004). For example, two previous studies conducted by our research group demonstrated that aging in rats is linked to high levels of fatty acid-binding protein 4 (a protein linked to adipose cells) and increased adipose cells in CT (Barin et al., 2017; Marqueti et al., 2018). These factors may compromise tendon structure leading to weakness (Marqueti et al., 2018). The finding of decreased adipose cells 28 days post injury could thus be a positive sign indicating ongoing recovery from the imbalances suffered by the GA injury. Additionally, decreased adipose cells in 28D may have contributed to lower overall thickness of the peritendinous sheath (**Figure 3**).

Some limitations of the present study should be highlighted, such as the inability to identify the cell types contributing to mRNA levels since gene expression analysis was conducted on the whole tendon. Moreover, the lack of immunoblot assay of essential proteins and immunofluorescence would be relevant to clarify adjacent mechanisms involved in tendon remodeling. We further note that due to the practical necessity of testing all samples in a single day at each time point, there is a risk for batch effects from day-to-day in biomechanical data. To address this, we performed tests as consistently as possible by a single operator and present data from all samples along with the means (**Figure 5**). While we have no evidence that a batch effect of relevant size occurred, we state this limitation here and look forward to the results of future similar studies. We next intend to evaluate gene expression, morphology, and biomechanical properties of muscle over time following tendon injury in a similar model to the present study.

CONCLUSION

In summary, our data demonstrate plasticity of the calcaneus tendon in response to cryolesion of the gastrocnemius muscle in rats. The results suggest that such an adverse muscle condition initiates a complex period of remodeling spanning at least 28 days. At 3 days following injury, we observed dysregulation of signaling pathways associated with collagen I and disrupted mechanical properties; at 14 days, there was reduced collagen content but increased invasion of blood vessels into the tendon proper and peritendinous sheath cells; and by 28 days, there was a dramatic rise in Young's modulus and gene expression of transcription factors related to ECM synthesis, remodeling, and repair. This study highlights the importance of muscle-tendon interactions and provides insight into their underlying mechanisms. Our results suggest that tendon may be susceptible to tendinopathies following skeletal muscle injury; future human studies may be warranted to investigate this potential association.

DATA AVAILABILITY

All data generated or analyzed in this study were displayed at a database repository Gene Expression Omnibus (GEO, NCBI) (Accession GSE131884) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE131884).

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Research Ethics Committee of the Catholic University of Brasilia, Brasília, Brazil (protocol number: 028/2015).

AUTHOR CONTRIBUTIONS

FB, JD, and RM conceived and planned the design of the experiments. FB, RM, CA, JA, and AD performed the experiment, analyzed the data, and designed the figures from biomechanical test. FB, GR, IS, AR, and RM performed the experiments, analyzed the data, and designed the figures of rest of experiments. FB, IS, GR, AS, JD, and RM interpreted the results and worked in the writing of manuscript. RM, OF, and JD involved in planning and supervised the work, also contributed to the design and implementation of the research. AS, AA, JD, OF, and RM provided critical feedback and analysis of the manuscript. All authors discussed the results and contributed to the final manuscript.

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Muscle-Tendon Interaction During Human Dolphin-Kick Swimming

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Without high impact forces, it is not clear how humans can utilize tendon elasticity during low-impact activities. The purpose of the present study was to examine the muscle-tendon behavior together with the electromyographic (EMG) activities of the vastus lateralis (VL) muscle during the human dolphin-kicking. In a swimming pool, each subject ($n = 11$) swam the 25 m dolphin-kicking at two different speeds (NORMAL and FAST). Surface EMGs were recorded from the VL and biceps femoris (BF) muscles. Simultaneous recordings of the knee joint angle by electro-goniometer and of the VL fascicle length by ultrasonography were used to calculate the muscle-tendon unit and tendinous length of VL (L_{MTU} and L_{TT}, respectively). In the dolphin-kicking, the stretching and shortening amplitudes of VL L_{MTU} did not differ significantly between the two kicking speed conditions. However, both stretching and shortening amplitudes of the VL fascicle length were lower at FAST than at NORMAL speed whereas the opposite was found for the VL L_{TT} values. At FAST, the contribution of the VL tendinous length to the entire VL_{MTU} length changes increased. The EMG analysis revealed at FAST higher agonist VL activation from the late up-beat (MTU stretching) to the early down-beat phases as well as increased muscle co-activation of VL and BF muscles from the late down-beat to early up-beat phases of dolphin-kicking. These results suggest that at increasing kicking speeds, the VL fascicles and tendinous tissues during aquatic movements can utilize tendon elasticity in a similar way than in terrestrial forms of locomotion. However, these activation profiles of VL and BF muscles may differ from their activation pattern in terrestrial locomotion.

Keywords: ultrasonography, vastus lateralis, electromyography, muscle fascicle, swim

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INTRODUCTION

In terrestrial locomotion, the actively stretched skeletal muscle can enhance the power and/or efficiency, following the stretch-shortening cycle (SSC) concept (cf. Komi, 1992, 2003). In running and jumping, for example, impact forces can be converted and then be utilized as elastic energy during the ground contact phase. With higher running speeds and drop heights, the stored and released elastic strain energy can be increased within the muscle-tendon unit (MTU) (e.g., Bosco et al., 1981; Ishikawa and Komi, 2004, 2008). In aquatic movements, however, this is likely not the case given the low impact load and slow movement conditions due to hydrodynamics, viscosity,

and buoyancy as compared to the movements on land (Grimston and Zernicke, 1993; Enoka, 2015). Therefore, it remains questionable whether humans can utilize elastic strain energy caused by muscle-tendon interactions to improve performance during relatively low impact movements such as in swimming. This information is important when considering the training strategies of elite swimmers.

For underwater animals such as dolphins and tunas, their muscles possess long tendons (Bennett et al., 1987; Shadwick et al., 1999; Alexander, 2002). However, other studies suggested that tendons may not play very important roles as energy-saving springs during swimming when they calculated the tendon compliance for fishes (Bennett et al., 1987; Alexander, 1997, 2002). Therefore, many animal studies of swimming mechanics and energetics have been focused on temporal relationship between the muscle activation and muscle strain cycle *in vivo* (Shadwick et al., 1999; Shadwick and Syme, 2008). Shadwick et al. (1999) used sonomicrometry and electromyography to record the length changes of muscle fascicles and muscle activity during the tuna swimming, and they also found that the onset of muscle shortening on each side of the body exactly coincides with the timing when the body bends toward that side. However, the inertial force (muscle activation) on the tail occurs prior to the muscle fascicle shortening at the extremes of its side to side movements (Shadwick and Syme, 2008). If these forces can stretch the tendon greatly during the stretching of entire muscle tendon unit, the elastic strain energy would be utilized during the tuna swimming.

It has not been investigated whether human muscle fascicles during swimming can behave similarly as those of underwater animals. In humans, there are many reports about muscle activities during swimming (Martens et al., 2015), but little has been reported on the muscle-tendon behavior during swimming together with muscle activities. Recent musculoskeletal ultrasonography make now possible to examine muscle fascicle behavior during dynamic human movements. The vastus lateralis muscle is one of the major muscles for knee extension-flexion movements and it is easily detectable during human dynamic movements. Therefore, the purpose of the present study was to examine the muscle-tendinous behavior together with muscle activities of VL during the human dolphin-kick of swimming at different kicking speeds. It is hypothesized that during the human dolphin-kicking, the muscle fascicles and tendon can perform a SSC action. At higher swimming speeds, the greater stretching and shortening of the vastus lateralis tendinous tissues should be observed during the human dolphin-kicking. Therefore, the tendon elasticity might play important roles during human movements not only on land but also under water conditions.

MATERIALS AND METHODS

Subjects

Eleven competitive male swimmers (butterfly and backstroke specialists) volunteered to participate in the study (**Table 1**). All subjects participated in swimming activities 5–6 times a week. This study was carried out in accordance with the recommendations of research guideline, the human Ethics Committee of Osaka University of Health and Sport Sciences with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the human Ethics Committee of Osaka University of Health and Sport Sciences (authorization n°13-28).

TABLE 1 | Physical characteristics of the subjects (mean ± SD).

Swimmer (n = 11)	
Age (years)	20.9 ± 1.5
Height (m)	1.72 ± 0.06
Body mass (kg)	66.4 ± 4.7
Thigh length (m)	0.376 ± 0.034
VL MTU length (m)	0.372 ± 0.034
VL tendinous tissue length (m)	0.306 ± 0.036
VL fascicle length (mm)	70.3 ± 6.9

Protocols

In an outdoor swimming pool (25 m), each subject was requested to swim 25 m underwater using the dolphin-kick (legs only) without breathing. After warming-up, the following devices were attached on the right leg: the surface electrodes for recording thigh electromyographic activities (EMG), a goniometer (SG150/W, Biometrics Ltd., UK) for the knee joint angle and an ultrasound probe (Prosound α10, Hitachi-Aloka Inc., Japan) for the VL muscle length changes. After preparation of the measurements, the subjects swam the dolphin-kicking at two different speeds (**Supplementary Material**). First, each subject was requested to swim the 25 m dolphin-kicking with maximum effort (FAST). After sufficient rest for about 10 min, each subject was requested to swim the submaximal 25 m dolphin-kicking (NORMAL: at 60% of the maximum dolphin-kicking speed). In this NORMAL condition, they were given a 25 m target time based on their 25 m dolphin-kicking with maximal effort. After measurements of dolphin-kicking, each subject performed isometric maximum voluntary contraction (MVC) measurements. MVCs for knee extension and flexion were performed twice, to replicate the amount of knee extension and flexion assumed during dolphin-kicking (at a joint angle corresponding to ∼60° of knee flexion). The greater MVC trial was adopted to normalize muscle activities of BF and VL to MVC, respectively.

Measurement Parameters

The Surface Electromyographic (EMG) Activity and Knee Joint Angle Recordings

Muscle activities were recorded from the VL and biceps femoris (BF) muscles of the right leg using bipolar surface electrodes (diameter 6 mm, inter-electrode distance 20 mm; Blue Sensor N-00-S/25, Ambu Medicotest A/S, Olstykke, Denmark) with a multi-telemeter system (sampling frequency 2 kHz, input impedance >10 MΩ, common mode rejection ratio >80 dB, time constant: 0.03 s; P-EMG plus, Oisaka electronic equipment, Japan). The electrode placements followed the SENIAM (Surface

ElectroMyoGraphy for the Non-Invasive Assessment of Muscles) guidelines (Hermens et al., 2000) as accurately as possible. The VL electrodes were placed slightly lateral to the muscle mid-belly to accommodate the ultrasound probe. Before electrode placement, the skin was cleaned with alcohol. EMG signals were amplified (×1,000). The knee joint angular movements were recorded using a flexible electro-goniometer (SG150/W, Biometrics Ltd., UK). The flexible electro-goniometer was mounted along the line from the tip of the trochanter to the knee rotation center and the line from the knee rotation center to the lateral malleolus tip to measure knee joint angles during dolphin-kicking. The EMG signals and joint angle data were stored simultaneously to a personal computer through an analog digital converter with a sampling frequency of 2 kHz (Power 1401; Cambridge Electronics Design Limited, Cambridge, UK).

Ultrasonography Recordings

Ultrasonography was applied to record the longitudinal images of the VL fascicle length in the upright position and during dolphin-kicking. An ultrasound probe, which weigh ∼130 g, was positioned over the VL muscle belly of the right leg to measure the fascicle length and pennation angle in the upright position as well as during the dolphin-kicking. The ultrasound probe with a 6 cm long linear array probe (scanning frequency: 13 MHz, image frequency: 117 Hz, Prosound α10, Hitachi-Aloka Inc., Japan) was used for all subjects. Similar to prior investigations during locomotion (Finni et al., 2001a,b; Kawakami et al., 2002; Ishikawa et al., 2003; Ishikawa and Komi, 2004), the ultrasound probe was positioned over the mid-belly (Blazevich et al., 2006) of the VL muscle and secured with a custom-made Styrofoam cast and wrapped tightly around the thigh to minimize any probe movement during the dolphin-kicking.

An electronic pulse was used to synchronize the EMGs, knee joint angle data and ultrasound data. At each kicking speed, 8–10 successive dolphin-kick cycles of the right leg were recorded once the swimming pattern was stabilized.

Data Analyses

One cycle of the dolphin-kicking includes two phases based on knee joint movement; the up- and down-beat kick phases. The up-beat and down-beat kick phases were determined by the transition point from the flexion and extension of the knee joint (**Figure 2**). Moreover, the up-beat and down-beat kick phases were divided equally into the early and late parts. From the 8–10 recorded dolphin-kick cycles, all of them were kept for the knee joint angle and EMG analyses whereas the ultrasonography analyses concentrated on 3 successive dolphin-kick cycles. The knee joint angle data was filtered with a Butterworth fourth-order filter (cut-off frequency 10 Hz). The obtained knee joint angles were used to calculate the instantaneous VL muscle-tendon unit length (VL L_{MTU}) with the model of Visser et al. (1990). The superior and inferior aponeuroses as well as a VL fascicle was identified and then digitized from each ultrasonographic image. The instantaneous length of VL tendinous tissues (VL L_{TT}), which was defined as the sum of the proximal and distal tendinous structures and aponeuroses, was calculated by subtracting VL fascicle length multiplied by the cosine of its pennation angle from L_{MTU} (e.g., Finni et al., 2000, 2001a,b, 2003; Ishikawa et al., 2003):

$$\text{VL } L_{TT} = \text{VL } L_{MTU} - \text{VL fascicle length} \bullet \cos\theta \quad (1)$$

where VL L_{TT} is the instantaneous length of VL tendinous tissues, VL L_{MTU} is the instantaneous VL muscle-tendon unit length, and θ is the VL pennation angle created by the VL fascicle line and its attachments on the aponeurosis lines. This allowed the calculation of their corresponding mean knee angular change as well as amplitudes of VL L_{MTU}, L_{TT}, and fascicle length changes. The reliability and reproducibility of the ultrasound method of fascicle length calculation has been reported in previous studies (Kawakami et al., 2002; Ishikawa et al., 2003; Giannakou et al., 2011). The normalized two-dimensional cross-correlation coefficient was used to show the reproducibility of the ultrasound images during the 1 cycle of dolphin-kicking between 2 cycles for each subject. The correlation coefficient for images during 1 cycle of dolphin-kicking was on an average 0.918 ± 0.001. The reliability of the ultrasound method of calculating the VL fascicle length was determined by calculating the coefficient of variation between each point during the dolphin-kicking. The mean value was 4.5 ± 0.3%.

The EMG signals were band-pass fourth-order filtered (25–450 Hz) and full wave rectified. These signals and joint angular data of 8–10 stable cycle kicks were averaged for each cycle to get the averaged time course data for each subject whereas the ultrasonography analyses concentrated on three successive cycles. Thereafter, the averaged EMG amplitudes (aEMG) were calculated individually and separately for the four different phases during the dolphin-kicking (see above definitions). The recorded EMG values for VL and BF muscles during swimming were, respectively, normalized relative to knee extension/flexion MVC EMG values for each subject to consider the muscle activation level for swimming.

Statistics

All values are presented as means ± standard deviations in the text and figures. Statistical analyses were performed using paired *t*-test for comparing the stretching and shortening amplitude of the VL LMTU, fascicle length and LTT between NORMAL and FAST conditions. For EMG data, normalized average EMG amplitudes were compared using two-way repeated measures ANOVA and Bonferroni *post-hoc* tests when applicable were used to examine the main effects of each parameter and interaction between the conditions (NORMAL and FAST) and phases (early and late up-beat kick phases, early and late down-beat kick phases). A criterion alpha level of $P < 0.05$ was used to determine statistical significance for all data. SPSS 23.0 software was used for statistical analyses.

RESULTS

The actual mean dolphin-kick speeds were 1.3 ± 0.1 m s^{-1} at FAST and 0.9 ± 0.1 m s^{-1} at NORMAL. The 1 cycle times of the dolphin-kicking for NORMAL and FAST were 647 ±

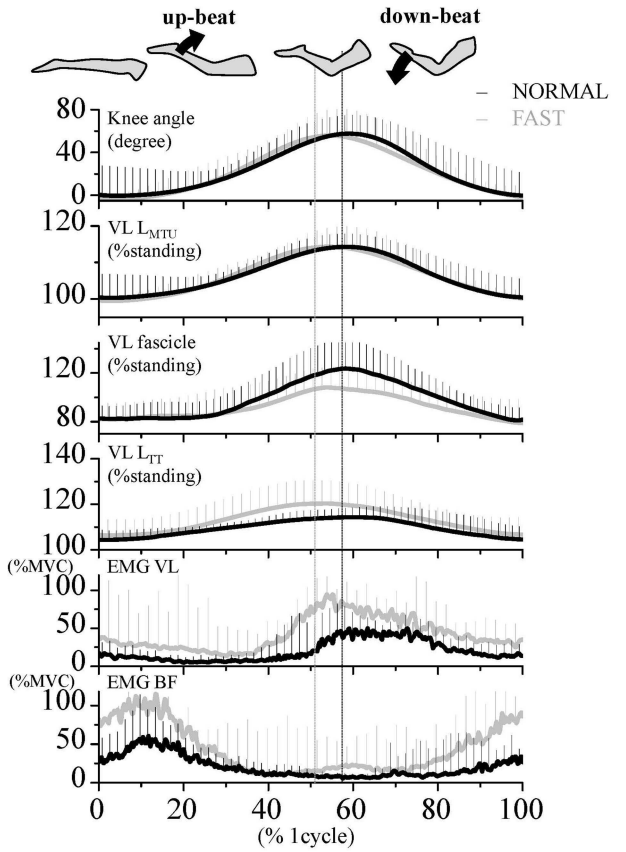

FIGURE 1 | Time course of the knee joint angle, length of muscle-tendon unit (L_{MTU}), fascicle and tendinous tissue (L_{TT}) of the vastus lateralis (VL) muscle, as well as EMG activities of VL and biceps femoris (BF) muscles during dolphin-kicking at the NORMAL (Black line) and FAST (Gray line) speeds. These lines are the group mean data over the entire 8 cycles of joint angles and EMG and the 3 cycles of length change data, respectively. Vertical error bars on the curves indicate standard deviation (+1 SD). The mean EMG values were expressed relatively to the EMG amplitudes of the maximum voluntary contraction (MVC). The vertical lines show the maximal point of knee flexion angle during the dolphin-kicking in both conditions. VL L_{MTU}, length of muscle-tendon unit; VL L_{TT}, length of VL tendinous tissues.

104 and 468 ± 46 ms, respectively. The separated phase times during the up-beat kick phase were 370 ± 76 and 235 ± 23 ms, respectively, and those during the down-beat kick phase were 278 ± 32 and 233 ± 31 ms, respectively. These times (1 cycle, up-beat and down-beat kick phases) were significantly shorter at FAST than at NORMAL ($p < 0.01$, respectively). The transition timing from the up-beat to down-beat kick phases of the 1 cycle dolphin-kicking was significantly shifted earlier in FAST (50.3 ± 2.9%) than in NORMAL (56.7 ± 3.3%) ($p < 0.001$).

During the dolphin-kicking, the amount of knee joint angular changes did not show any significant differences between both kicking speed conditions (**Figures 1**, **2A**). In the length behavior, the VL fascicle length and VL L_{TT} as well as VL L_{MTU} at both NORMAL and FAST during the dolphin-kicking underwent a stretching and shortening behavior from the up-beat to down-beat kicking (**Figure 1**). The stretching and shortening amplitudes of the VL L_{MTU} did not show any significant differences between NORMAL and FAST (**Figure 2B**). However, the relative stretching and shortening amplitudes of the VL fascicle length were smaller at FAST than at NORMAL (ΔVL fascicle stretching: $p < 0.01$, ΔVL fascicle shortening: $p < 0.05$, respectively) (**Figure 2C**). Inversely, the stretching and shortening amplitudes of the VL L_{TT} were greater at FAST than at NORMAL speed (ΔVL L_{TT} stretching and shortening: $p < 0.05$, respectively). Although the stretching and shortening speeds of the VL fascicle length did not show any significant differences between NORMAL and FAST, the stretching and shortening speeds of the VL L_{TT} were faster at FAST than at NORMAL (ΔVL L_{TT} stretching: $p < 0.01$, ΔVL L_{TT} shortening: $p < 0.05$, respectively, **Figure 3**).

The VL EMG activity at NORMAL increased gradually during the late up-beat kick phase and then reached its maximal muscle activation during the early down-beat kick phase (**Figures 1**, **4**). On the other hand, the antagonist BF EMG activity was the highest at NORMAL speed during the early up-beat kick phase (**Figures 1**, **4**). At increasing kicking speeds, the VL aEMG activity of each phase was significantly greater at FAST than at NORMAL (**Figure 4**). The BF aEMG activity during the early and late up-beat as well as the late down-beat kick phases were also significantly higher at FAST than at NORMAL.

DISCUSSION

The results of the present study clearly show that the VL muscle fascicles and tendinous tissues as well as the MTU present a SSC behavior during the knee joint flexion and extension during human dolphin-kicking. At faster speeds of the dolphin-kicking, the stretching and shortening amplitudes of the VL fascicle length were decreased and inversely, those of the VL L_{TT} were increased. The contribution of VL tendinous tissues to the entire VL MTU length changes increased at the faster speed of the dolphin-kicking. Therefore, not only on land but also under water, the efficient muscle force output prevents the increasing speeds of the muscle fascicle stretching and shortening. The increased utilization of the VL tendinous elasticity is considered as the common muscle-tendon strategy to enhance power output for faster human movements, such as running, jumping, and swimming. In the muscle activation patterns, however, not only the VL activation from the late up-beat (MTU stretching) to the early down-beat kick phases, but also specific muscle co-activation between VL and BF from the late down-beat to early up-beat kick phases of dolphin-kicking may play important roles to enhance kicking speeds.

SSC Type of Muscle-Tendon Behavior During Human Dolphin-Kicking

In previous animal studies, the muscle stretching was calculated from body kinematics and/or EMG patterns during aquatic movements (e.g., Hess and Videler, 1984; Rome et al., 1993; Katz and Shadwick, 1998). They presumed that muscles perform

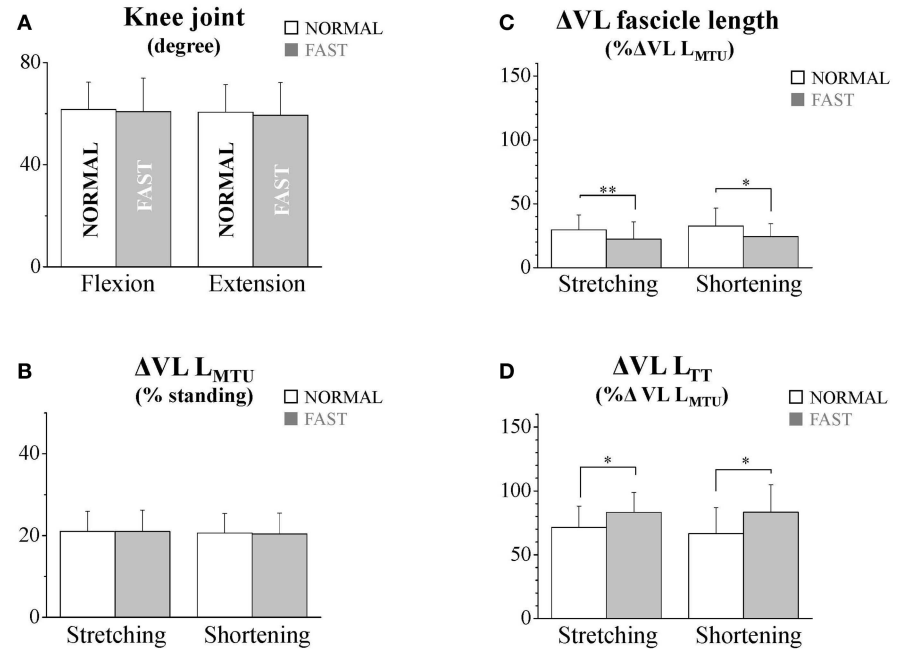

FIGURE 2 | The flexion and extension amplitudes of the knee joint **(A)**, the stretching and shortening amplitudes of the vastus lateralis (VL) muscle-tendon unit **(B)**, fascicles **(C)**, and tendinous tissues **(D)** at NORMAL and FAST speeds during the dolphin-kicking. The knee flexion and extension amplitudes were calculated from the maximal knee joint extension to the maximal flexion (up-beat) and from the maximal knee joint flexion to that maximal extension (down-beat) during the dolphin-kicking. The stretching (up-beat kicking) and shortening (down-beat kicking) amplitudes were calculated at NORMAL and FAST, respectively. The error bars show the standard deviations for all subjects (+1 SD). * and ** indicate significant differences between the NORMAL and FAST speed conditions at $p < 0.05$ and $p < 0.01$, respectively.

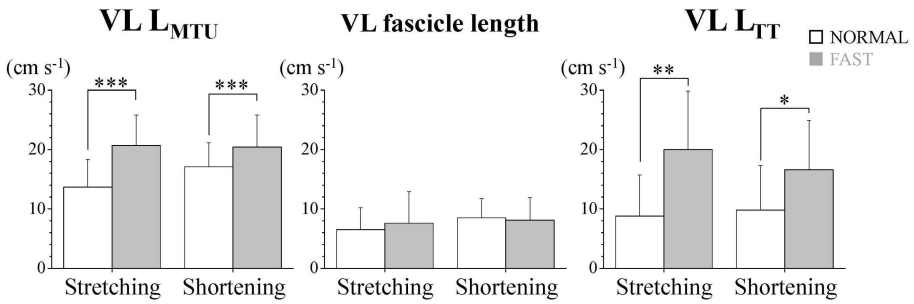

FIGURE 3 | Stretching and shortening speeds of the muscle-tendon unit (VL L_{MTU}), fascicle and tendinous tissue (VL L_{TT}) of the vastus lateralis muscle (VL) during dolphin-kicking. The error bars show the standard deviations for all subjects (+1 SD). *, **, and *** indicate significant differences between the NORMAL and FAST speed conditions at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

predominantly positive work in animal swimming. However, such muscle work behavior during swimming would not necessarily be efficient. In human dolphin-kicking, the stretching and shortening behaviors of the VL muscle fascicles, tendinous tissues and MTU were observed in the same manner to running and jumping (Finni et al., 2001a,b; Ishikawa et al., 2003, 2005). At increasing kicking speeds, the stretching and shortening speeds of VL L_{MTU} and L_{TT} increased with the increased L_{TT} stretching and shortening amplitudes, although the speeds of VL fascicle length were unchanged and the amplitudes of VL fascicle length were decreased. These muscle fascicle and tendinous tissue interactions for enhancing performance of swimming are in line with those of previous studies in terrestrial locomotion (e.g., Ishikawa and Komi, 2008). Therefore, even in aquatic movements, the utilization of the VL tendinous elasticity would play an important role to enhance performance during the human dolphin-kicking. These may also explain the elastic behavior of connective tissues during the tuna swimming (Shadwick and Syme, 2008).

Specific Muscle Behavior and Activation Profiles During Human Dolphin-Kicking

There is still a question as to whether the behavior of the MTU during low impact load swimming is the same as in high

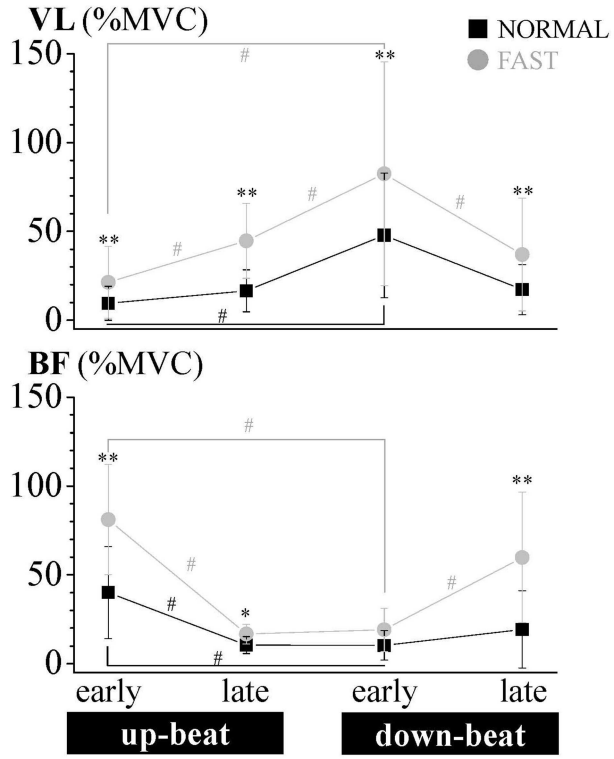

FIGURE 4 | Relative aEMG comparison of the vastus lateralis (VL) and biceps femoris (BF) muscles for each phase of the dolphin-kicking between the NORMAL and FAST speed conditions. The aEMG of the 8 cycle data for each subject were averaged for each phase. The aEMG values were expressed as the relative values to the maximum voluntary contraction (MVC) values. The error bars show the standard deviations (± 1 SD) for all subjects. ** and * show significant differences between conditions at $p < 0.01$ and $p < 0.05$, respectively. # shows significant differences between phases at $p < 0.05$.

impact load jumps on land. Following the SSC concept (e.g., Komi, 1992, 2000), the pre-programed and stretch reflex muscle activation of agonist muscles is considered as playing important roles for performance enhancements through neuromuscular potentiation and utilization of elastic energy in drop jumps and hopping.

At both NORMAL and FAST speeds, not only VL but also BF was activated during the late down-beat phase in FAST. This BF antagonist muscle action (eccentric) can not only decelerate the knee extension at the end of the range of motion (ROM) but also enhance the kicking movements of the leg segment, causing the hyperextension of the swimmer knee. In the following early up-beat phase, both VL and BF were additionally activated at faster speed. BF then functions as an agonist (concentrically) to initiate knee flexion and inversely VL functions as an antagonist (eccentrically) to prevent knee flexion and fascicle lengthening. This increased antagonist VL activation concomitantly with the increased BF activation during the knee flexion at FAST can result in an increased muscle stiffness and function as the preprogramed muscle activation in SSC for complementing the low impact load dolphin kicking. On the other hand, a very small amount of co-activation occurred during the late up-beat and early down-beat phases. These different activation profiles among phases may be related to the gravitational loading and dominant kicking direction. Such co-activation during the transition phase has been observed in animal swimming (e.g., Altringham and Elleby, 1999), where the eccentric activation of an antagonist muscle results in an increased muscle stiffness and storage of elastic energy that serves to decelerate the joint at the end of the ROM. In the up-beat phase, the knee flexion and MTU stretching occurred gradually together with the BF and VL muscle activations at the early up-beat phase at FAST. In the following late up-beat phase, VL but not BF was highly activated leading to less VL fascicle lengthening as an eccentric action to decelerate knee flexion and to increase the stretching of the VL tendinous tissues efficiently in a similar fashion to the terrestrial locomotion (e.g., Ishikawa and Komi, 2008). Finally, during the early down-beat phase, VL continued to be active but functioned then as an agonist to initiate the knee extension. The reduced shortening velocity of VL muscle fascicles at FAST during the down-beat phase may have the advantage to produce force and power at the MTU level during the down-beat phase (VL shortening phase).

Thus, our results clearly showed that the VL stretching of the muscle fascicles and tendinous tissues did not start from the early up-beat but from the late up-beat kick phases in both conditions, increasing the contribution of VL tendinous tissues to the entire VL MTU length changes to enhance the kicking speed. However, at the low impact load swimming, not only the VL activation during the late up-beat phase but also the co-activated VL and BF muscles during the transition from the down-beat to up-beat phases can play important roles to compensate for the low impact loads and knee joint stiffness level due to hydrodynamics during swimming. Therefore, to enhance the power output of the MTU shortening during swimming, the high joint stiffness due to the co-activation from the late down-beat phase and high VL eccentric activation during the late up-beat phase can stretch the VL tendinous tissues effectively to enhance the kicking speed, even when the MTU stretching amplitudes were same in both conditions.

Methodological Limitations

There are certain points in the methods that need to be addressed. The present study is perhaps the first one in which fascicle length (VL) and EMG activities were recorded simultaneously during human underwater swimming. We have measured only one knee extensor and flexor muscles during dolphin-kicking. However, other muscles at the knee, ankle, and hip joints can also contribute to dolphin-kicking and should be measured in the future studies. Secondly, the attached wired ultrasound probe and goniometers in the present study may influence the dolphin-kicking speeds. However, the maximal kicking speed value (1.3 ± 0.1 m s-1) for the FAST condition was similar in the speeds previously reported for male and female national level swimmers (1.2 ± 0.1 m s^{-1}) (Connaboy et al., 2016) and for Olympian swimmers (1.5 m s^{-1}) (Arellano et al., 2002; Loebbecke et al., 2009). The dolphin-kicking speeds in the FAST condition could

be still high enough to examine the dolphin-kicking movements competitive swimmers.

CONCLUSION

During human dolphin-kicking, the VL fascicles and tendon present a stretch-shortening action behavior. To enhance the swimming speeds of dolphin-kick, the tendinous elastic energy can play important roles for an efficient MTU force output. With faster swimming speed, the VL tendinous elastic energy was stored more from the late up-beat to early down-beat phases of dolphin-kicking. Even in this aquatic condition, a unique muscle co-activation of VL and BF muscles from the late down-beat to early up-beat phases of dolphin-kicking can keep and enhance the high VL muscle force and stiffness level during the up-beat phase of dolphin-kicking.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of research guideline, the human Ethics Committee of Osaka University of Health and Sport Sciences with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the human Ethics Committee of Osaka University of Health and Sport Sciences (authorization n°13-28).

AUTHOR CONTRIBUTIONS

KS, MI, and PK designed the experiment and wrote the manuscript. KS, TS, YD, and RN verified the analytical methods, performed the analyses, and interpreted the results. All authors performed the experiments, discussed the results, and commented on the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.01153/full#supplementary-material

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frontiers in Physiology

ORIGINAL RESEARCH
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Effects of High-Intensity Interval Training and Isoinertial Training on Leg Extensors Muscle Function, Structure, and Intermuscular Adipose Tissue in Older Adults

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We compared the effects of aerobic high-intensity training (*HIT*) and isoinertial resistance training (*IRT*) on the strength, mass, architecture, intermuscular adipose tissue (*IMAT*) quality, and neuromuscular activation of the quadriceps in elderly subjects. Twelve healthy men (69.3 ± 4.2 years; 77.8 ± 10.4 kg; 1.72 ± 0.05 m) were exposed to 8 weeks of *HIT* (7 × 2-min cycling repetitions at 90% of $\dot{V}O_{2peak}$, 3 times/week) and, after 4 months (detraining), to *IRT* (4 × 7 maximal concentric–eccentric knee extensions, 3 times/week). Before and after trainings, we measured knee extension isometric (T_{MVC}) and dynamic (T_C) maximal concentric torque, anatomical cross-sectional area (*ACSA*) at 25, 50, and 75% of femur length, quadriceps volume (*Vol*), *IMAT,* pennation angle (θ_p) of the fibers from the *vastus lateralis*, and voluntary activation (*%Act*). T_{MVC} and T_C were significantly larger only after *IRT* (P = 0.008); *IRT* was able to elicit a greater increase of *ACSA* than *HIT*; *Vol* increases similarly and significantly after *HIT* and *IRT* (P = 0.003–0.001); *IMAT* at 50% of femur length decreased after both *HIT* and *IRT* (P = 0.001–0.003); physiological cross-sectional area (*PCSA*) was larger after *IRT* than before (P = 0.025); specific torque did not change throughout the study (45.5 N cm^{-2} ± 12.0); *%Act* of the quadriceps was significantly affected only by *IRT* (P = 0.011). Both *HIT* and *IRT* are able to elicit beneficial modifications of muscular mass, architecture, and quality (reducing *IMAT*) in elderly subjects in connection with an amelioration of strength. *HIT* and *IRT* caused a homogeneous increase of *ACSA* and of *Vol* of the quadriceps. *PCSA* increases, but specific strength per unit of *PCSA* did not change. The increases of functional torque seemed to be attributed to a parallel increase of *%Act* and muscle hypertrophy only after *IRT*. Data suggest that *IMAT* may be a prominent indicator to track metabolic-dependent activity and skeletal muscle quality.

Keywords: aging, high-intensity interval training, isoinertial resistance training, muscle volume, muscle architecture, muscle activation, intermuscular adipose tissue

INTRODUCTION

In elderly people, the loss of muscle mass and strength has a negative impact on their daily life autonomy, balance, and gait (Narici and Maffulli, 2010). Sarcopenia (the progressive loss of muscle mass and strength with age), however, has also functional and metabolic consequences: the progressive decrease of lean body mass is mirrored by the decay of resting metabolic rate (Narici and Maffulli, 2010), and it also implies a decrease of daily physical activities and of total energy expenditure (Vaughan et al., 1991; Goran and Poehlman, 1992). Both these factors predispose elderly people to accumulate visceral and total body fat (Kohrt and Holloszy, 1995) and to develop poor insulin sensitivity and increased post-prandial hyperglycemia.

Furthermore, also, the quality of the muscles decays in the elderly because of the substantial increase of the so-called intermuscular adipose tissue (*IMAT*) (Marcus et al., 2010; Zoico et al., 2010). Unlike subcutaneous adipose tissue, *IMAT*, which might be viewed as a peripheral ectopic fat depot, surrounds and infiltrates muscle groups with which it shares a direct vascular connection. This anatomic relationship is analogous to that of visceral liver and abdominal fat, suggesting that *IMAT* might have a functional negative influence on skeletal muscle metabolism analogous to that of visceral adipose tissue on liver metabolism (Durheim et al., 2008). In spite of its role, thigh *IMAT* has not been widely studied.

It is commonly observed that long-term heavy strength training increases anatomical cross-sectional area (*ACSA*) and muscle volume (*Vol*) mainly because of preferential Type-II fiber hypertrophy (Tesch, 1988). Yet, there seems to be a selective growth within the muscles involved in training (Housh et al., 1992; Narici et al., 1996) that may depend on the magnitude of their activation due to the load imposed by the mechanic gain of each single muscle belly (Folland and Williams, 2007). However, the data are not completely consistent, since it has not been clarified whether selective hypertrophy is more pronounced, for instance, in the region of the maximal *ACSA* of the quadriceps (Häkkinen et al., 2000) or in the more distal and proximal regions of the muscles (Narici et al., 1996), also as a consequence of different training modalities and, therefore, regional loads. Differential adaptations are likely to alter the moment of inertia of the thigh and the functional consequences (Earp et al., 2015). The architectural changes must be properly taken into account when we aim to estimate the possible changes of specific tension elicited by training, since force must be normalized by the so-called physiological cross-sectional area (*PCSA*) and not by the *ACSA*. In this regard, it is not clear whether specific torque (i.e., the torque per unit of *PCSA*) may change following resistance training. In addition, any decrease in *IMAT* content brought about by training would, however, modify the specific strength calculated as the ratio between torque and *ACSA*.

In the elderly, the progressive loss of muscle mass and strength (sarcopenia) is associated with metabolic alterations. Furthermore, the peripheral changes that compromise gas exchanges contribute to the progressive decrease in the maximum cardiovascular oxygen transport observed in aging. It is therefore ecological to propose strength training. It is useful to counteract the loss of muscle mass by promoting or enhancing the beneficial adaptations induced also by aerobic training on the main risk factors leading to metabolic syndrome. Even though traditional calisthenic workout (whole-body exercises, 3 sets of 10–15 reps, 3 days/week) has been described to improve health-related parameters as biomarkers (Tomeleri et al., 2016) and body fat (Cunha et al., 2017), resistance training is mainly prescribed in elderly people to promote the increase of muscle strength and mass (Hurley et al., 1994; Hunter et al., 2004). Since resistance training is highly effective when concentric and eccentric contractions are repeatedly applied (Berg and Tesch, 1994; Franchi et al., 2017), the so-called isoinertial resistance training (*IRT*) performed with the YoYo® ergometer, which implements the flywheel principle and is able to generate resistance force during both the lengthening and shortening actions of the contraction thanks to eccentric overload (Tesch et al., 2017), seems to be a promising and effective way to induce a fast increase of muscle mass and strength also in elderly people (Maroto-Izquierdo et al., 2017).

The improvement in muscle strength following resistance training is also the result of beneficial neuromuscular adaptions. The main neurological adaptations, which seem to establish already in the early phases of training, include a decrease of the activation of the antagonist muscles and enhanced agonist muscle activation due to increased motor recruitment, firing frequency, and synchronization of the motor units (Gabriel et al., 2006; Folland and Williams, 2007). By using the interpolated twitch technique to measure the level of muscular activation during maximal, voluntary isometric contraction (Shield and Zhou, 2004), several studies have indeed shown that increased muscle activation follows strength training (Shield and Zhou, 2004). Even if a high-intensity interval training (*HIT*) program induced beneficial muscular neural adaptations in young adults (Vera-Ibañez et al., 2017), no evidence exists of any amelioration in muscle activation in the elderly population.

HIT has gained popularity as a safe and efficient exercise method with the potential to influence several health-related parameters: in various populations, *HIT* has been shown not only to increase maximal oxygen consumption ($\dot{V}O_{2max}$) but also to decrease fat mass and increase insulin sensitivity (Gibala et al., 2006; Bruseghini et al., 2015). Moreover, it has been observed that *HIT* can lead to an increase of skeletal muscle oxidative and buffering capacity, muscle protein synthesis, and mitochondrial biogenesis (Scalzo et al., 2014). Despite the aforementioned data that suggest a potential positive impact of *HIT* on muscle remodeling and growth, there are limited data on the effect of *HIT* on muscle mass, strength, architecture, and quality in elderly (Astorino et al., 2011). *HIT* has been described to contrast

Abbreviations: *%Act*, percent neuromuscular activation; θ_p, pennation angle of muscle fibers; *ACSA*, anatomical cross-sectional area; *BMI*, body mass index; *HIT*, high-intensity interval training; *IMAT*, intermuscular adipose tissue; *IRT*, isoinertial resistance training; L_f, fascicle length; *MVC*, maximal voluntary contraction; $\dot{V}O_2$, oxygen uptake; $\dot{V}O_{2max}$, maximal oxygen uptake; *PCSA*, physiological cross-sectional area; *QF*, *quadriceps femoris*; *RF*, *rectus femoris*; *RT*, resistance training; T_C, T_{MVC}, Maximal concentric and isometric muscular torques; T_m, muscle thickness; $T_{MVCcorr}$, corrected maximal isometric force exerted on the patellar tendon calculated from T_{MVC} at 90° of knee flection; *VI*, *vastus intermedius*; *VL*, *vastus lateralis*; *VM*, *vastus medialis*; *Vol*, muscle volume.

the progressive loss of muscle mass (Harber et al., 2012) and 8 weeks of *HIT* were followed by the decrease of the percentage of adipose tissue and by the hypertrophic adaptation of the muscle involved in training (Bruseghini et al., 2015). If *HIT* induces also a substantial decrease of *IMAT*, this would result in a substantial improvement of muscle quality and function (Addison et al., 2014). In this regard, no clear data exist on the efficacy of *HIT* to reduce *IMAT* in elderly, active, healthy men, even though it has been shown that moderate physical activity (e.g., walking) was able to prevent the increase of *IMAT* in older subjects (Goodpaster et al., 2008).

On the basis of all these premises, we tested the hypothesis that muscular mass-volume, quality, morphology, and function can be increased/improved by both types of training intervention. Therefore, we evaluated the effects of 8 weeks of *HIT* and *IRT* on muscle quality, morphology, strength, and neuromuscular activation of healthy, elderly men. Also, we test the hypothesis that structural adaptations would differ between training modalities because of the differences in muscular and regional activation. The results would help to obtain a deeper insight into the specific effectiveness of these two types of training on muscular quality, morphology, and function in elderly subjects considering that these features have a substantial impact on the metabolic and locomotor roles of the skeletal muscle.

Therefore, we aimed to assess (i) whether *HIT* elicited any substantial increase of muscle strength and mass; (ii) whether muscle hypertrophy elicited by training was selectively more pronounced in specific areas of the quadriceps; (iii) if *HIT* and *IRT* were able to induce significant amelioration of the quality of the muscles and, in turn, modification of the strength-to-*ACSA* ratio; (iv) whether muscle morphology was substantially modified by the two training interventions; and (v) the relative contribution of muscle hypertrophy and muscular increased activation in eliciting the observed increments of strength.

MATERIALS AND METHODS

Subjects

Twelve moderately active Caucasian men (*age*: 69.3 ± 4.2 years, range, 65–75; *body weight*: 77.8 ± 10.4 kg; *height*: 1.72 ± 0.05 m; *BMI*: 26.5 ± 2.8 kg m^{-2}; *IPAQ score*: 4333 ± 1750 MET min $week^{-1}$) were recruited through local advertisements in the Verona, Italy, metropolitan area and volunteered to participate in the study. Main physiological and health-related outcomes (e.g., $\dot{V}O_{2max}$, body composition) have been previously published for this sample of participants (Bruseghini et al., 2015; Tam et al., 2018). All the subjects had filled in the IPAQ questionnaire and then they underwent a preliminary medical examination to evaluate exclusion criteria [abnormal *EKG* at rest and during exercise, uncontrolled hypertension, diagnosis of cardiovascular, respiratory and metabolic diseases, moderate-severe renal failure, neurological and orthopedic diseases limiting mobility and exercise, anti-coagulants and anti-aggregant therapy, alcohol and drug abuse and common contraindications to *MRI* (i.e., pacemakers, metallic clips)] and pathological responses to exercise. The study was conducted in accordance with ethical standards, the provisions of the Declaration of Helsinki, and national and international guidelines. The protocol and the methods of the study were approved by the Regional Review Board (approval on June 18, 2013), and written informed consent was obtained from each subject before entering the study.

Experimental Design

A two-factor within-subject design was planned in which each subject received all the combinations of treatment that originated by crossing the two factors: one fixed factor was training modality (two levels, *HIT* and *IRT*); the second fixed factor was time (two levels, *Pre-* and *Post*-training) in which all subjects were exposed to all the two conditions (Keppel and Wickens, 2004). All the subjects were evaluated four times: before training for baseline values (*Pre-HIT*) and immediately after 8 weeks of *HIT* (*Post-HIT*), after 12 weeks of recovery before *IRT* (*Pre-IRT*), and, finally, after 8 weeks of resistance training (*Post-IRT*). Before the first data collection, a familiarization session was conducted, during which the experimental procedures were thoroughly explained and a simplified version of them was carried out.

During each experimental session, the tests were performed in the morning, at the same time of the day and in the environmentally controlled conditions, on three consecutive days: on the first day, the main anthropometric measurements were carried out and the $\dot{V}O_{2max}$ of each subject was measured. The second day was devoted to functional strength test and ultrasound scan acquisition. The third day was dedicated to *MRI* scans. Twenty-four hours before the tests, participants abstained from strenuous physical activity and alcohol and caffeine consumption.

Training Protocols

The subjects were asked to perform each supervised training session at the same time of the day on alternate days. Compliance to training was high with subjects in each training period completing all of the exercise sessions. No injuries or health disorders were reported during the exercise program, and no modification in the planned protocol had to be introduced. Between and during *HIT* and *IRT* sessions, the subjects were asked to maintain their habitual lifestyle: to evaluate physical activity, all subjects wore a portable monitor SenseWear Armband Mini (BodyMedia, Inc., Pittsburgh, United States) continuously for a 1-week period (Mackey et al., 2011). This has been done 1 month before each training period and during both training periods.

High-Intensity Interval Training

Volunteers trained three times a week for 8 weeks. Training consisted of seven 2-min bouts of cycling exercise (915 E, Monark, Varberg, Sweden) at 85–95% of individual $\dot{V}O_{2max}$ interspersed by 2-min recovery intervals at about 40% of $\dot{V}O_{2max}$ (Buchheit and Laursen, 2013a,b). Each series was preceded by 10 min of active warm-up. The entire supervised training session lasted from 45 to 50 min, including the post-training cooling-down phase. The mechanical workloads related to the percentage of $\dot{V}O_{2max}$ were calculated using the individual oxygen consumption/load ratio of the warm-up before the

incremental test and created using the oxygen consumption values measured in the last minute of each load. Heart rate/load (*HR/W*) ratio was also computed in order to control responses to exercise and to adjust workloads every 14 days, according to changes in the *HR/W* relationship assessed during three submaximal workloads at steady state.

Isoinertial Resistance Training

Resistance exercise was performed on a seated knee extension flywheel ergometer (Berg and Tesch, 1994) (YoYo Technology AB, Stockholm, Sweden) 3 times a week for 8 weeks. Each supervised session consisted of four sets of seven maximal, coupled concentric extensions and eccentric flexions of the knee from about 90° to 160°–170° knee joint angle. Subjects received verbal encouragement to push as harder as they could and direct feedback was provided during exercise by shoving force production. The increase in the maximum force applied during each training session has made it possible to enhance and adapt the workload constantly during the 8 weeks of training. The sets were interspersed by 3-min rest periods and initiated immediately after performing two submaximal actions. Each exercise session was preceded by 10 min of active warm-up, including three sets of seven submaximal actions with progressively increased effort. Training was performed using a polymer flywheel (4.2 kg). Each exercise session, including warm-up and rest periods, was completed in about 30 min.

Anthropometry and Maximal Oxygen Uptake

Body weight and stature were measured and *BMI* was also calculated. $\dot{V}O_{2max}$ was measured using a metabolic cart (Quark b^2, Cosmed, Rome, Italy) at the end of incremental ramp tests to exhaustion on a cycle ergometer (Excalibur Sport, Lode, Groningen, Netherlands), as described in detail previously (Bruseghini et al., 2015).

Muscle Volume, Cross-Sectional Area, and *IMAT*

To determine the volumes and *ACSA* of the total *quadriceps femoris (QF)*, *rectus femoris* (*RF*), *vastus lateralis* (*VL*), *vastus intermedius* (*VI*), and *vastus medialis* (*VM*), *MRI* scans in a 1.5-T GE scanner (General Electric, Milwaukee, WI) were obtained following the protocol described by Trappe et al. (2001). Briefly, a coronal scout scan [repetition time/echo time (TR/TE) 5 300/14 ms, field of view 48 cm, 256 × 160 matrix] of five slices 5 cm thick with 5-mm spacing was completed to establish orientation of the femur. Then, interleaved transaxial images of 1 cm thick (TR/TE 633/20 ms, field of view 274 × 480 mm, 256 × 256 matrix) were obtained along the entire length of the femur. The procedure has already been discussed previously (Bruseghini et al., 2015) and only the most salient details will be explained here. Analyses of the magnetic resonance images of dominant limb began with the first proximal slice not containing gluteal muscle and continued distally to the last slice containing *RF* (Castro et al., 1999). The average *ACSA* (cm^2) was taken as the average of all the analyzed slices for an individual muscle and determined for the *RF*, *VL*, *VI*, and *VM* and summed for the total *QF*. *ACSA* was drawn manually in correspondence of slice obtained at 75, 50, and 25% of the length between the greater trochanter to the upper border of the patella (*LF*). The volume of muscle tissue per slice was calculated by multiplying the *ACSA* area by the inter-slice distance. The volumes of each of the *QF* components were calculated as the sum of all corresponding slice volumes. The volume of the quadriceps (*Vol*) was then computed as the sum of the single muscular volume.

MRI scans of dominant limb at 75, 50, and 25% femur length were examined to determine *IMAT* and subcutaneous adipose tissue using SliceOmatic image analysis software (version 4.2; TomoVision, Montreal, Quebec, Canada). *IMAT* was defined as adipose tissue area visible between quadriceps muscle groups. The gray-level intensity (threshold value) of the adipose tissue in the subcutaneous adipose tissue region was determined. This value was reduced by 20% to identify the quadriceps *IMAT* threshold (Rossi et al., 2010).

Muscular Strength

Isometric and dynamic strength produced by the knee extensors of the dominant limb was evaluated with an isometric–isokinetic dynamometer (CMSi Cybex Humac Norm Dynamometer, Stoughton, MA, United States) at 90° of knee angle during maximal voluntary contraction in isometric condition (T_{MVC}) and during concentric (T_C) isokinetic contractions at an angular velocity of 120° s^{-1}. Before the strength test, the subjects completed 10 min of warm-up exercise on a stationary bike, and they performed several practice trials while sitting on the reclining chair of dynamometer. The lower part of the leg was strapped to the end of the lever arm of the dynamometer and the center of rotation of the knee was aligned with the axis of the dynamometer. Three maximal trials (30 s of rest was provided between each trials) were performed for each condition with 3 min of recovery between each condition (Connelly and Vandervoort, 2000; Power et al., 2013). Visual feedback was provided to participants and verbal encouragement was standardized throughout both testing protocols (McNair et al., 1996). The highest torque values were recorded for further analysis.

Muscle Architecture

Real-time B-mode ultrasonography (ACUSON P50 ultrasound system, 12L5 linear probe) was used to measure fascicle pennation angle (θ_p) and fascicle length (L_f) of the *VL*. Participants were sitting with the knee angle fixed at 90° (Raj et al., 2012). Images were obtained at mid-belly of the dominant *VL* muscle by using a linear-array probe. The probe was positioned perpendicular to the dermal surface of the *VL* muscle and oriented along the median longitudinal plane of the muscle. Mid-belly was defined as the point along the median longitudinal axis of the muscle at 50% of the distance between the proximal and distal apexes of the myotendinous junctions. The center of

the probe was aligned to this position. The probe was coated with a water-soluble transmission gel to provide acoustic contact without depressing the dermal surface. Three images at rest were obtained within the same experimental session in each individual (Narici et al., 2003). Scans were analyzed with an open source software OsiriX (Pixmeo, Geneva, Switzerland). *VL* muscle thickness was defined as the distance between the superficial and deep aponeurosis. θ_p was measured as the angle between the muscle fascicles and the deep aponeurosis. L_f was measured as the length of a fascicle between its insertions at the superficial and deep aponeurosis. Where the fascicles extended beyond the recorded image, L_f was estimated from muscle thickness (T_m) and θ_p using the following equation (Raj et al., 2012):

$$L_f = T_m \times \sin \theta_p^{-1} \quad (1)$$

Then, the *PCSA* of the quadriceps (*PCSA*) was calculated as follows (Gans, 1982):

$$PCSA = Vol \times \theta_p \times L_f^{-1} \quad (2)$$

θ_p and L_f measured on the *VL* were assumed to be representative of the mean θ_p and L_f of the entire quadriceps (Erskine et al., 2009). Quadriceps specific tension (T_s) was subsequently calculated using the following formula:

$$T_s = T_M VCcorr \times PCSA^{-1} \quad (3)$$

where $T_{MVCcorr}$ is the tension in N calculated from T_{MVC} at 90° of knee flexion corrected by the patellar tendon moment arm length obtained from the literature (Smidt, 1973; Narici et al., 1992).

Neuromuscular Activation

To determine the level of voluntary muscle activation and contractile proprieties of the dominant quadriceps muscle and biceps muscle of the arm (control condition), the interpolated twitch technique was used (Shield and Zhou, 2004). During experiments on lower limb, the participants sat in a standardized position with hip and knee at 90° of flexion, on a customized testing system's chair and tightly secured to it with hip and torso straps. During experiments on upper limb, the elbow of the subjects was accommodated in a standardized position on a customized dynamometer placed on a table; the dominant forearm was then positioned vertically and connected to the load cell rigidly attached to the customized testing system. Electrical stimulation was administered via two 5 cm × 10 cm self-adhesive electrodes, placed distally (anode) and proximally (cathode) over the quadriceps (Kufel et al., 2002). The *quadriceps* and the *biceps brachii* were stimulated in a relaxed state with 50-mA pulses of 100 μs, which were increased in 30-mA increments (Digitimer High Voltage Stimulator model DS7A, Digitimer Ltd., Welwyn Garden City) until no further increase in twitch force was observed. This current was used 2 min later to elicit a single twitch during three maximal voluntary contractions (*MVC*) lasting 5 s each and a second twitch in the resting state 5 s after the *MVC*.

Force was measured by means of a calibrated load cell (DBBE, Applied Measurements Ltd., Aldermaston, Reading, United Kingdom) connected to a non-compliant strap that was placed around the subject's dominant leg just superior to the ankle malleoli and around the forearm close to the wrist of the dominant arm. Torque signals and electrical stimuli collected with the help of PowerLab data acquisition (PowerLab 16/35 AD Instruments Ltd., Australia) at a sampling frequency of 1 kHz and analyzed by Labchart 6.0 software (AD Instruments Ltd., Australia).

Voluntary activation of the stimulated muscles (*%Act*) was quantified applying the following equation:

$$\%Act = [1 - (superimposedtwitch/controltwitch)] \times 100 \quad (4)$$

where the superimposed twitch is the force increment noted during a maximal voluntary contraction at the time of stimulation and the control twitch is that evoked in the relaxed muscle (Shield and Zhou, 2004).

Statistical Analysis

All values in the text are presented as mean ± standard deviation. Sample size was determined using G*Power software (ver 3.1.9.2) (Faul et al., 2007) to ensure there was sufficient power (1–β = 0.80) to detect significant differences within factors. Normality of data distribution was evaluated by means of the Shapiro–Wilk test (StatPlus: mac Version v6, AnalystSoft, CA, United States). When criteria for normality were not met, inferential analysis was always performed on the log-transformed data. Overall analysis of the data was carried out according to Keppel and Wickens (2004) for two-fixed factors within-subjects design with Training type (*HIT* and *IRT*) and Time (*Pre* and *Post*) as fixed. In particular, (i) *F* values were calculated taking into account the possible violation of sphericity by using the correction of the degree of freedom, as suggested by Geisser and Greenhouse; (ii) effect size in terms of population variability was evaluated by computing ω^2, which expresses the variability of the effect over the sum of that variability and the error variability and total variability that affects it; (iii) single planned contrasts time (*Pre* vs. *Post*) and Training modalities, *HIT* vs. *IRT* were evaluated; (iv) effect size (*d*) of the differences between the contrasted values was also calculated. Calculations were carried out using an Excel spreadsheet (MO 2010, Microsoft Corp. Seattle, WA, United States) programed for this purpose.

The differences between the percent increase of the cross-sectional areas of the quadriceps between *Pre*- and *Post*-interventions were evaluated by using a 2-ANOVA analysis for repeated measurements; *post-hoc* analysis was carried out between families of pairwise comparisons by using the Šidák–Bonferroni procedure to correct for the family-wise Type I error. Multiple linear regressions between a dependent variable and two independent explanatory variables were calculated by using least squares approach (Motulsky and Christopoulos, 2004).

Correlation analyses were conducted on *ACSA*, *IMAT*, *%Act*, with Pearson's product–moment correlation, and correlation coefficients (*r*) were classified as small ($0.1 < r \leq 0.3$), moderate ($0.3 < r \leq 0.5$), high ($0.5 < r \leq 0.7$), very high ($0.7 < r \leq 0.9$), and almost perfect ($r > 0.9$) (Hopkins et al., 2009).

RESULTS

The results have been reported synthetically (mean ± SD) in **Supplementary Table S1** for greater clarity and commented in the following sessions.

Cross-Sectional Areas

HIT was followed by a significant increase of *ACSA* at all the three femur lengths [at 25%: plus 3.09 cm^2 ± 1.38; (P = 0.001; 95% CI of diff: 2.21–4.0; d: 2.24); at 50%: plus 2.27 cm^2 ± 2.52 (P = 0.010; 95% CI of diff: 0.67–3.87; d: 0.90); at 75%: plus 2.65 cm^2 ± 3.04 (P = 0.011; 95% CI of diff: 0.72–4.58; d: 0.87)] (**Figure 1**).

Also, *IRT* was followed by a significant increase of *ACSA* at all the three evaluated % of femur length [at 25%: plus 3.19 cm^2 ± 1.24; (P = 0.001; 95% CI of diff: 2.40–3.99; d: 2.57); at 50%: plus 3.03 cm^2 ± 3.04 (P = 0.005; 95% CI of diff: 1.10–4.96; d: 1.00); at 75%: plus 3.40 cm^2 ± 3.21 (P = 0.004; 95% CI of diff: 1.36–5.44; d: 1.06)] (**Figure 1**).

ANOVA analysis revealed a significant effect of factor *Time* on the increase of *ACSA* at 25% of femur length (F = 0.001; ω^2 = 0.59), 50% of *LF* (F = 0.001; ω^2 = 0.64), and 75% (F = 0.002; ω^2 = 0.25). At 25% and at 75% of femur length, also an effect of factor *Training* was present (F = 0.028; ω^2 = 0.10): at 25%, *ACSA* at *Post-IRT* was larger than at *Post-HIT* (P = 0.024; d = 0.753); at 75%, *ACSA* at *Post-IRT* was larger at *Post-HIT* (P = 0.008; d = 0.929) than at *Pre-HIT* (P = 0.011; d = 1.291). In none of the cases, however, significant interactions between factors were present.

The highest quadriceps *ACSA* was found at 50% of femur length. However, for both trainings, the percent increases of quadriceps *ACSA* at the 3% of femur length were rather homogeneous and they were not significantly different. The percent increases of quadriceps *ACSA* at the 3% of femur length were not different between *HIT* and *IRT*.

Finally, the percent increase of quadriceps *ACSA* observed after trainings at the three levels of femur length was negatively and moderately related with the absolute area at *Pre-HIT* and *Pre-IRT*: r = -0.37; P = 0.001; n = 72.

Volume of the Quadriceps

ANOVA analysis revealed a significant effect of time on the increase of *Vol* (F = 0.001; ω^2 = 0.848). *Vol* was significantly larger at *Post-HIT* than at *Pre-HIT*: plus 42.2 cm^3 ± 38.3 (P = 0.003; d = 1.11), and at *Post-IRT* than at *Pre-IRT*: plus 68.2 cm^3 ± 38.3 (P = 0.001; d = 1.40) (**Figure 2**).

The percent increase of the total *Vol* of the quadriceps was not significantly different between *HIT* and *IRT*.

Intermuscular and Subcutaneous Adipose Tissues

ANOVA analysis on the data of *IMAT* at 50% of femur length was performed after log transformation of the data as they failed the test for assessing normal distribution. The analysis revealed a significant effect of time (F = 0.001; ω^2 = 0.78) and of training (F = 0.001; ω^2 = 0.66).

There was a progressive decrease of the *IMAT* area during the study. In particular, *IMAT* was significantly lower at *Post-HIT* than at *Pre-HIT* (P = 0.001; d = 1.57). As the analysis was performed on log-transformed data, the 95% CI of the ratio between the *Pre-HIT* and *Post-HIT* were calculated: 0.70–1.15. *IMAT* significantly decreased also after *IRT* in respect to *Pre-IRT* (P = 0.003; d = 1.08), the 95% CI of the ratio being equal to 0.51–1.49. *IMAT* after the strength training was also significantly smaller than at *Post-HIT* (P = 0.001; d = 1.64) with 95% CI of the ratio equal to 0.42–1.40) (**Figure 3**).

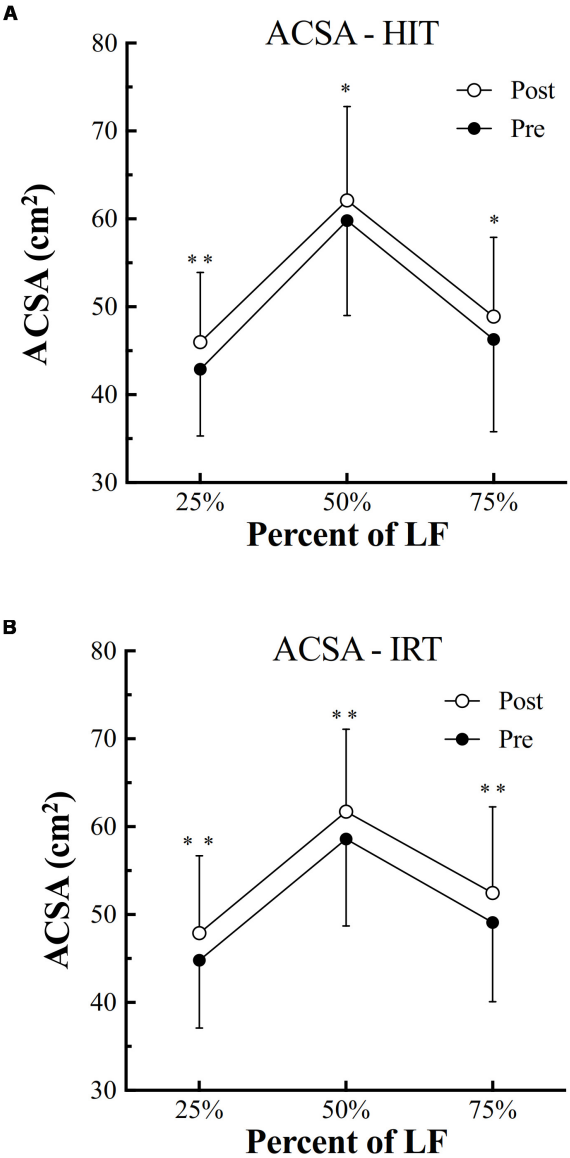

FIGURE 1 | Anatomical cross-sectional area (*ACSA*, cm^2) of the quadriceps assessed before (filled symbols) and after (empty symbols) high-intensity interval training (*HIT*, **A**) and isoinertial resistance training (*IRT*, **B**) at 75, 50, and 25% of the length between the greater trochanter to the upper border of the patella (*LF*) (*P < 0.05; **P < 0.01).

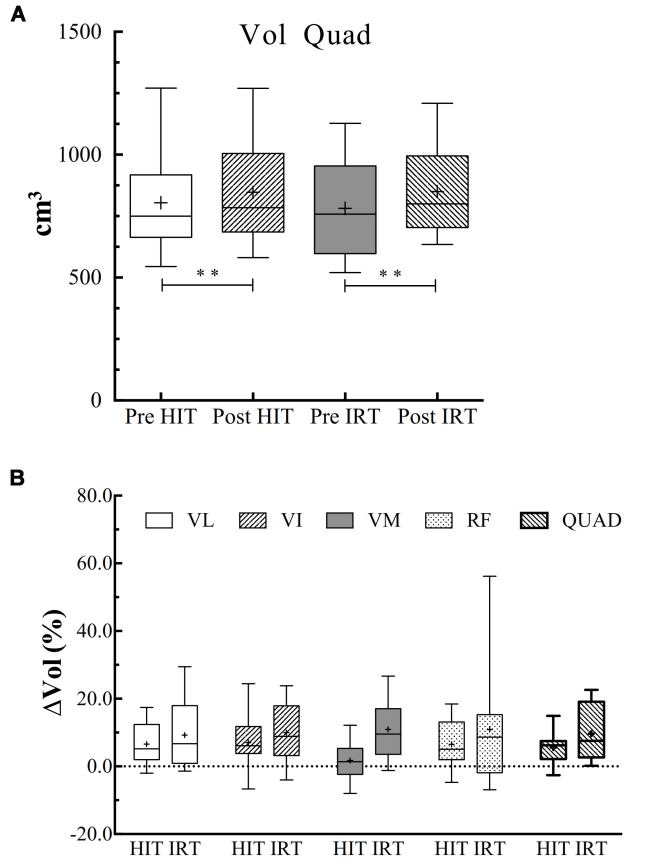

FIGURE 2 | (A) Box and whiskers plot of the volumes of the quadriceps (*Vol*, cm^3) before and after *HIT* and *IRT*. Asterisks indicate the significant difference between *Pre-* and *Post*-conditions (**$P < 0.01$). **(B)** Box and whiskers plots of the percent increases (*Vol*, %) of the entire quadriceps and of each of its belly observed during *HIT* and *IRT*. The box extends from the 25th to 75th percentiles. The line in the middle of the box is plotted at the median and "+" at the mean. Whiskers range from a Min to Max value.

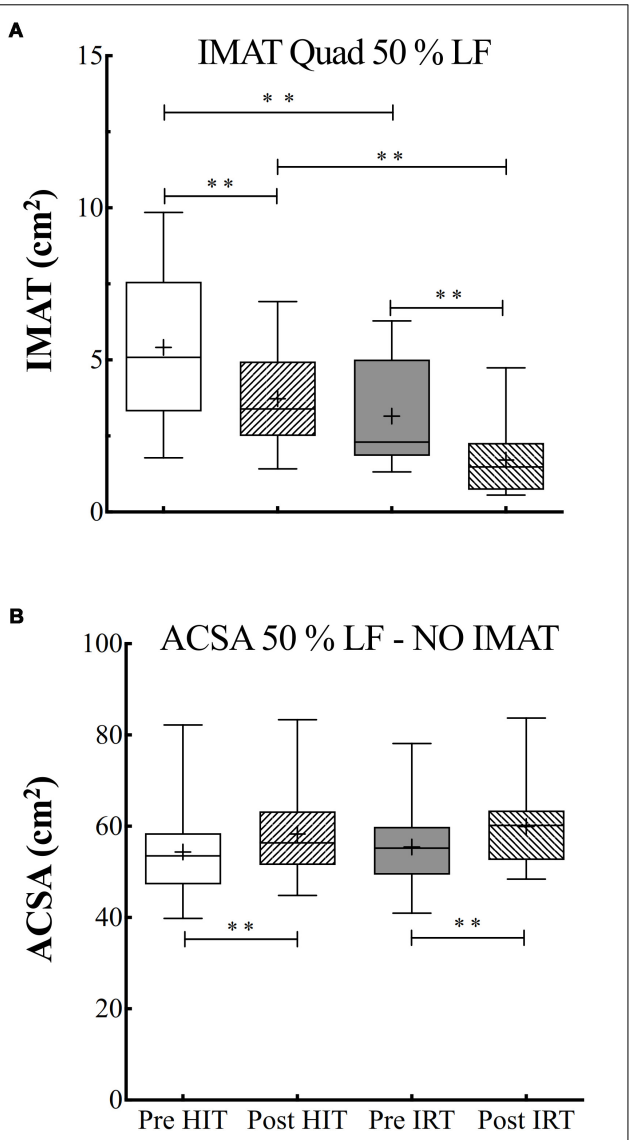

FIGURE 3 | (A) Box and whiskers plots of intermuscular adipose tissue (*IMAT*, cm^2). **(B)** Box and whiskers plots of *ACSA* (cm^2) measured at 50% of the length between the greater trochanter to the upper border of the patella (% *LF*) excluding *IMAT*. Lines and asterisks indicate the significant differences between the mean values (**$P < 0.01$). The box extends from the 25th to 75th percentiles. The line in the middle of the box is plotted at the median and "+" at the mean. Whiskers range from a Min to Max value.

Also, for subcutaneous adipose tissue at 50% of femur length, it was possible to show an effect of time ($F = 0.001$; $\omega^2 = 0.63$): subcutaneous adipose area significantly decreased after *HIT* in respect to the initial control condition ($P = 0.001$; $d = 1.31$, 95% CI of diff: −5.97 to −7.73). An effect of the type of training was also evident ($F = 0.001$; $\omega^2 = 0.66$). In addition, significant interactions between time and training effects were also demonstrated ($F = 0.003$; $\omega^2 = 0.56$), as the differences between *Pre-HIT* and *Post-HIT* values were significantly different from those observed before and after *IRT* ($P = 0.010$; $d = 0.99$).

The absolute and percent changes of *ACSA* at 50% of femur length were also calculated net of *IMAT* contribution. The subtraction of *IMAT*, however, did not alter the pattern of the changes observed after the two training interventions: *ACSA* at *Post-HIT* was significantly larger than at *Pre-HIT*: plus 3.95 $cm^2 \pm 3.17$ ($P = 0.001$; *d*: 1.25; 95% CI of diff: 1.93–5.96) and at *Post-IRT* than at *Post-IRT*: plus 4.47 $cm^2 \pm 2.63$ ($P = 0.001$; *d*: 1.697; 95% CI of diff: 2.79–6.14). Interestingly, the percent increases of total *ACSA* neglecting *IMAT* were significantly larger than the ones measured including *IMAT* both for *HIT* (7.71% ± 6.21 vs. 4.00% ± 4.29; $P = 0.001$, 95% CI of discrepancy from 0: 2.1–5.3) and for *IRT* (8.38% ± 5.47 vs. 5.65% ± 6.14; $P = 0.006$, 95% CI of discrepancy from 0: 0.97–4.48) (**Figure 3**).

For the sake of completeness, also the analysis of *IMAT* at 25% and at 75% of femur length was carried out after log transformation of the data. The data generally confirmed the ones obtained at 50%: the analysis revealed a significant effect of time ($F = 0.001$; $\omega^2 = 0.70$) at 25% of femur length. In details, *IMAT* was significantly lower at *Post-HIT* than at *Pre-HIT* ($P = 0.003$; $d = 1.12$) and at *Post-IRT* than at *Pre-IRT*

(P = 0.008; d = 0.92). At 75% of femur length, however, *IMAT* turned out to be significantly lower only after *IRT* than at *Pre-IRT* (P = 0.001; d = 1.244).

Finally, the net decrement of *IMAT* observed in the two training interventions was highly correlated (r = –0.71) with the initial absolute value of *IMAT*.

Muscle Torque

Only *IRT* training was followed by a significant increase of T_{MVC} measured at 90° of knee joint flexion: plus 11.5 N m ± 17.1 (P = 0.040; d = 0.67): the percent of increase amounted to 7.0% ± 9.8 (**Figure 4**).

Training significantly affected T_C at 120° s^{-1} (F = 0.014; ω^2 = 0.137), but isokinetic strength was only significantly larger after *IRT* than before *IRT*: plus 8.8 N m ± 13.0 (P = 0.008; d = 0.93). For strength values, the baseline conditions *Pre-HIT* are comparable to baseline conditions *Pre-IRT* (**Figure 4**).

Percent increases of T_C were not linearly related with the corresponding initial torque values (P between 0.050 and 0.705). They ranged, on average, from 2.44 to 10.4% (grand mean at 120° s^{-1}: 6.6% ± 11.3).

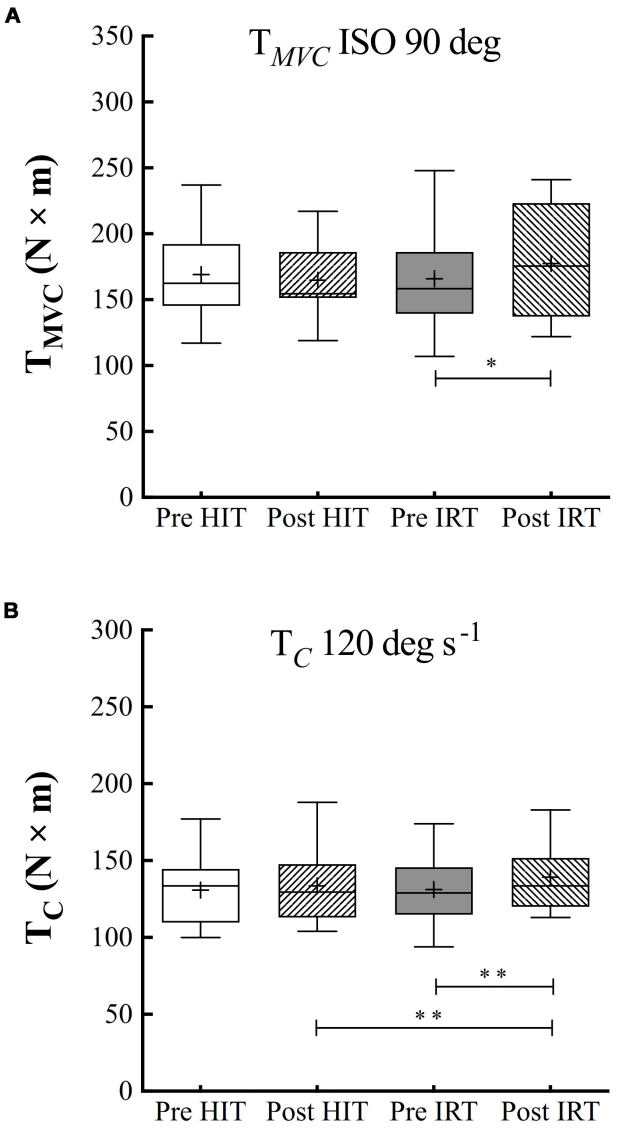

FIGURE 4 | Box and whiskers plots of isometric torque at 90° of knee flection **(A)** and isokinetic concentric torque (T_C, N × m) at 120° s^{-1} of angular velocity **(B)**. Lines and asterisks indicate the significant differences between the mean values (*P < 0.05; **P < 0.01). The box extends from the 25th to 75th percentiles. The line in the middle of the box is plotted at the median and "+" at the mean. Whiskers range from a Min to Max value.

Pennation Angle, PCSA, and Specific Torque

ANOVA analysis revealed a significant effect of time (F = 0.019; ω^2 = 0.410) and of training (F = 0.001; ω^2 = 0.119) on θ_p, which turned out to be significantly greater after *HIT* than before (P = 0.001; d = 1.93) and after *IRT* than before strength training (P = 0.004; d = 1.03) (**Figure 5**).

PCSA at 50% of femur length was significantly affected by training (F = 0.041; ω^2 = 0.083), but was only larger at *Post-IRT* than at *Pre-IRT* (P = 0.025; d = 0.741) (**Figure 5**).

However, when specific isometric strength was calculated as the ratio between strength and *ACSA*, we obtained a different pattern if we corrected or did not correct *ACSA* for *IMAT* (**Figure 6**). When *IMAT* is included, torque per square centimeter of *ACSA* did not change during the study, and its grand average amounted to 59.8 N cm^{-2} ± 7.1, 95% CI 61.8–57.7. When *ACSA* was corrected for the contribution of *IMAT*, torque per squared cm of *ACSA* at *Post-HIT* (60.8 N cm^{-2} ± 7.5) was smaller than at *Pre-HIT* (66.4 N cm^{-2} ± 6.1), P = 0.007, 95% CI of the difference from 0: 1.9–9.3 N cm^{-2}. Conversely, it did not change from *Pre-IRT* (63.8 N cm^{-2} ± 5.6) to *Post-IRT* (63.0 N cm^{-2} ± 9.1). Finally, specific strength measured as the ratio between T_{MVC} and *PCSA* remained constant during the study, and its grand average amounted to 45.5 N cm^{-2} ± 12.0, 95% CI 49.0–42.0.

Muscular Activation

ANOVA analysis for the two fixed effects demonstrated that %*Act* of the quadriceps was affected by training (F = 0.019; ω^2 = 0.119), being larger at *Post-IRT* than at *Pre-IRT* (P = 0.011; d = 0.897) (**Figure 7**). Arm activation remained identical throughout the study.

Moreover, %*Act* of the quadriceps was negatively correlated with the absolute levels of *IMAT* both before (r = 0.21, small correlation) and after (training (r = 0.53, high correlation).

The percent increases of isokinetic and isometric torque selected as explanatory variables (Y) were fitted to a multiple linear regression with the percent increases of *ACSA* at 50% of femur length (X_1) and of %*Act* (X_2) as predictive variables for both *HIT* and *IRT* conditions, forcing the functions to pass through the origin of the axis:

$$HIT: Y = X_1 \times 0.568(0.252) + X_2 \times 0.041(0.037); \quad (F = 3.78; P = 0.03 \tag{5a}$$

$$IRT : Y = X_1 \times 0.634(0.209) + X_2 \times 0.152(0.075); \quad (F = 9.45; P = 0.0001); \tag{5b}$$

where Y, X_1, and X_2 represent the percent increases of torque, *ACSA*, and %*Act*, respectively.

However, in *HIT*, only the percent of increase of *ACSA* significantly predicted the increase of strength ($t = 2.25$; $P = 0.03$); in *IRT*, both percent of increases of *ACSA* ($t = 3.03$; $P = 0.004$) and %*Act* ($t = 2.031$; $P = 0.048$) significantly predicted the percent increase of Torque.

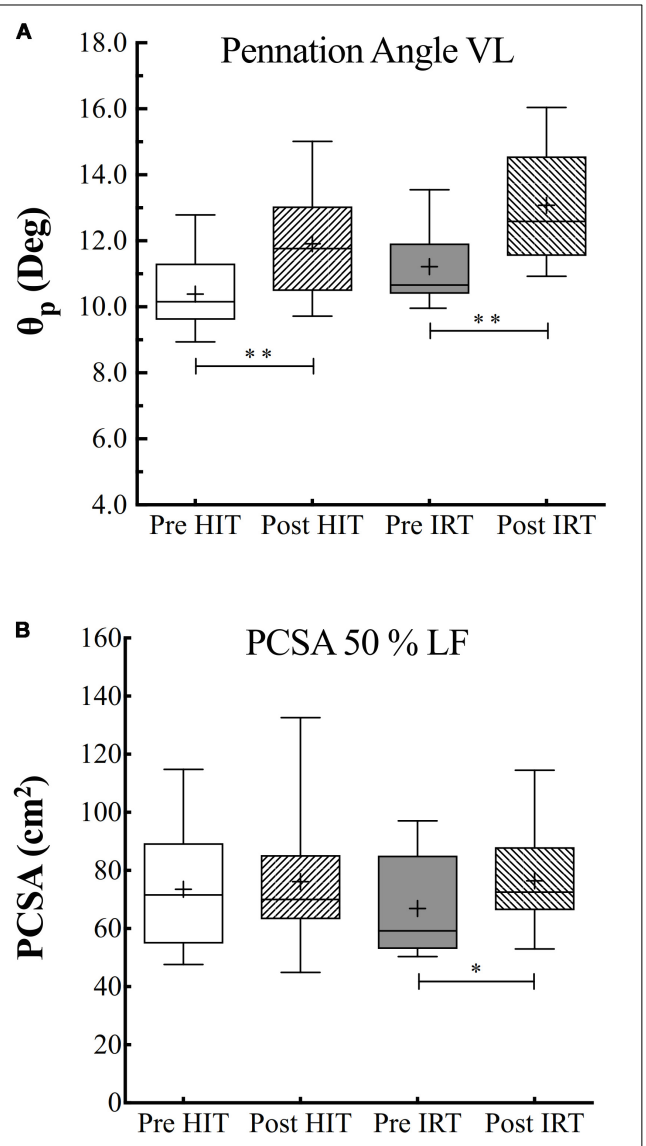

FIGURE 5 | Pennation angle (θ_p, °) of the fibers of *vastus lateralis* **(A)** and physiological cross-sectional area (*PCSA*) of quadriceps in cm^2 **(B)** assessed before (*Pre*) and after (*Post*) *HIT* and *IRT*. Lines and asterisks indicate the significant differences between the mean values (*$P < 0.05$; **$P < 0.01$). The box extends from the 25th to 75th percentiles. The line in the middle of the box is plotted at the median and "+" at the mean. Whiskers range from a Min to Max value.

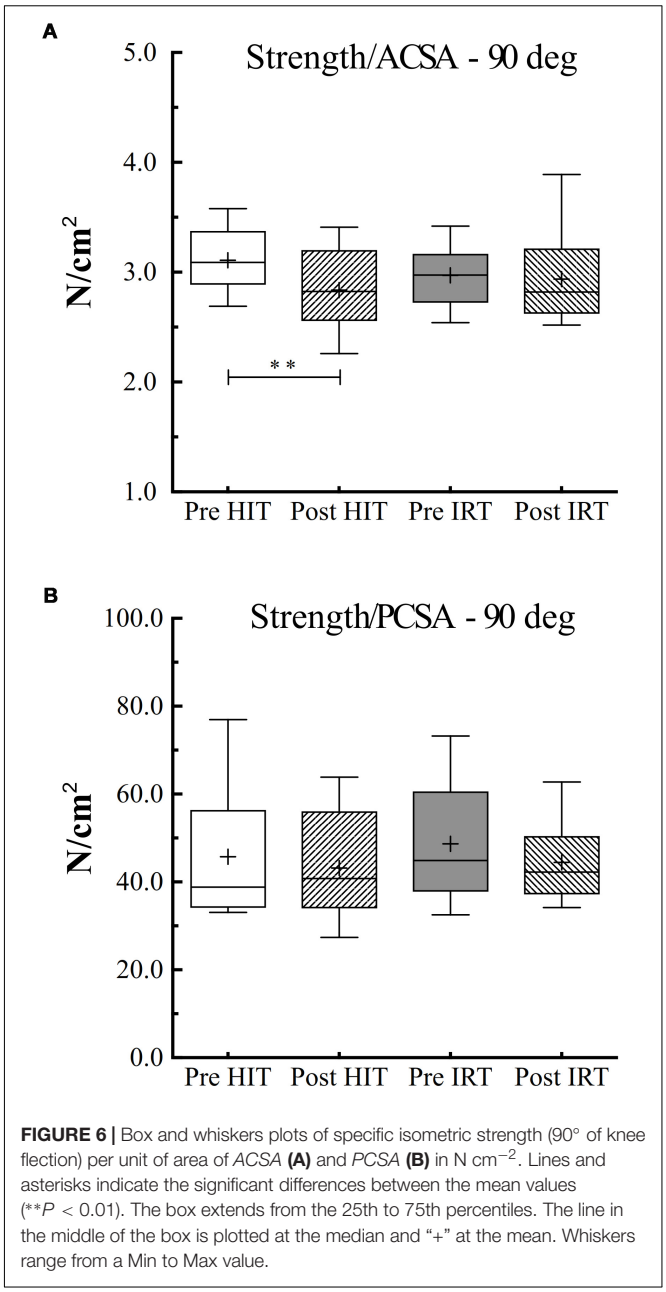

FIGURE 6 | Box and whiskers plots of specific isometric strength (90° of knee flection) per unit of area of *ACSA* **(A)** and *PCSA* **(B)** in N cm^{-2}. Lines and asterisks indicate the significant differences between the mean values (**$P < 0.01$). The box extends from the 25th to 75th percentiles. The line in the middle of the box is plotted at the median and "+" at the mean. Whiskers range from a Min to Max value.

DISCUSSION

The purpose of this investigation was to compare the effects of *HIT* and *IRT* on muscular strength, mass, morphology, muscle quality, i.e., *IMAT*, and neuromuscular activation in a group of active, healthy elderly subjects.

The main findings showed that:

i) T_c and T_{MVC} turned out to be significantly larger only after *IRT*; *ACSA* of quadriceps significantly increased both after *HIT* and after *IRT* at three evaluated femur lengths; the efficacy of training was also confirmed by the analysis of the changes of *Vol*;

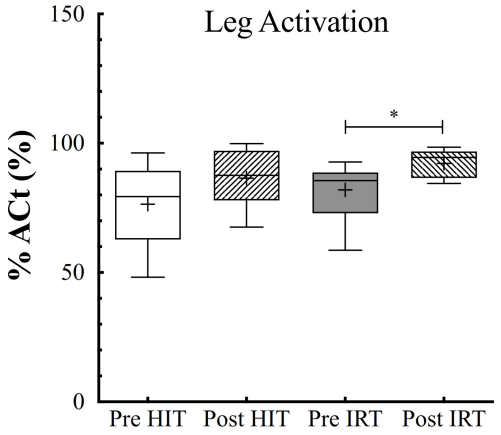

FIGURE 7 | Box and whiskers plots of percent of voluntary activation *(%Act*, %) of the lower limb extensors assessed before (*Pre*) and after (*Post*) *HIT* and *IRT*. Lines and asterisks indicate the significant differences between the mean values (*$P < 0.05$). The box extends from the 25th to 75th percentiles. The line in the middle of the box is plotted at the median and "+" at the mean. Whiskers range from a Min to Max value.

ii) *IMAT* at 50% of femur length decreased after both trainings, in particular after *IRT*, and moreover, although the removal of *IMAT* did not change the pattern of the percent change of *ACSA* after trainings, it turned out to be larger than the ones calculated including *IMAT* area;
iii) θ*p* increased both after *HIT* and *IRT*; however, *PCSA* turned out to be larger only after *IRT*; the changes in *PCSA* and in torque resulted in constant value of specific strength per unit of *PCSA*;
iv) *%Act* of the quadriceps was only greater after the *IRT* and the increments of strength observed after *IRT* seem to be predicted by the consensual increases of *ACSA* and %*Act*.

Therefore, both *HIT* and *IRT* seemed to be able to induce significant and remarkable changes in muscle mass, morphology, and quality, but strength turned out to be significantly and positively affected only by *IRT*. The overall analysis of physical activity during the intervention period shows that subjects were able to maintain the same lifestyle, and they have not changed their *total daily energy expenditure* during the two training programs (before *HIT*: 2318 ± 315 kcal/day; during *HIT*: 2255 ± 314 kcal/day; before *IRT:* 2253 ± 243 kcal/day; during *IRT*: 2344 ± 220 kcal/day).

Muscle *ACSA* and Volume

Both *HIT* and *IRT* were able to induce muscle hypertrophy, since *ACSA* increased at all the three femur lengths, as shown in **Figure 1**, and *Vol* was augmented after the two training interventions (**Figure 2**). The findings after *HIT* are in agreement with the ones reported by other investigators (Harber et al., 2012; Estes et al., 2017) who showed a significant increase of *Vol* in older and young men after moderate-to-vigorous aerobic training. The percent increase reported in those studies is of the same order of magnitude as the one found in the present investigation, i.e., 5.5% ± 4.6. Harber et al. (2012) attributed the observed hypertrophy to the specific increase of *ACSA* of *MHC Type 1* fibers.

Strength training has been shown to induce remarkable increases of muscle mass in elderly subjects (Cadore et al., 2014) and 10 to 12 weeks of heavy resistance training resulted in increase of *ACSA* of the quadriceps ranging from 6% (Kraemer et al., 1999) to 11–14% (Häkkinen et al., 2000). In the present study, the average percent increase of *ACSA* at 50% of *LF* was 5.7% ± 6.4, a value that might be explained by the shorter duration of the intervention and by the different training protocols applied in the studies. In this respect, *IRT* with eccentric overload has been claimed to be more effective and capable to induce larger gains in muscle mass and strength (Maroto-Izquierdo et al., 2017) than traditional weight lifting. However, a recent review (Vincens-Bordas et al., 2018) has refuted this conclusion, highlighting that the available data do not allow us to conclude that *IRT* is superior to traditional weight lifting and gravity-dependent strength training in increasing muscle mass and strength. Yet, it is worth noting that the majority of the available data were collected on young or adult men and women and conclusive data concerning elderly individuals on the comparison of the two training modalities are scarce.

The largest *ACSA* was found at 50% of femur length in agreement with the findings of Narici et al. (1996). However, in contrast with their findings, and with the ones of Mangine et al. (2018), the increase of *ACSA* found after the two trainings was uniform at the three levels of femur length. The discrepancy between the data obtained in this investigation and the ones reported in the quoted paper deserves, of course, a comment. First, our study concerned elderly subjects, whereas others investigated adult young volunteers. We know that different increases of *ACSA* have been found between young and elderly subjects (Kraemer et al., 1999), although this has not been consistently confirmed (Cadore et al., 2014). Therefore, we may speculate that the less pronounced response of the elderly subjects to the training stimulus may have somehow blunted and made less evident the selective growth of the four bellies of the quadriceps. Secondly, *IRT*, with its eccentric overload, may lead to a massive activation of all the bellies of the quadriceps, thus overriding the limitation of the gravity-dependent training that may induce activations of different magnitude as a consequence of the different loads imposed to each singular muscle by their mechanical gains (Folland and Williams, 2007).

Muscular morphological adaptations are also paralleled by substantial changes in muscle architecture. There is a general agreement that the pennation angle (θ_p) of the muscle fibers increases with hypertrophy (Kawakami et al., 1993; Folland and Williams, 2007). The increase of θ_p would allow a larger packing of fibers for the same *ACSA* and lead to the increase of the *PCSA*, i.e., the area perpendicular to the line of application of the force produced by the fibers. However, an increase of θ_p would bring about a decrease of the force applied to the tendon because the angle between the fibers and the line along which the force is projected decreases. Yet, it can be geometrically demonstrated that if θ_p stays below 45°, its increase is compensated by the increase of *PCSA* so that an augmentation of force results after training (Alexander and Vernon, 1975). Indeed, it has

been shown that an increase of θ_p from 8.0° to 10.7° (+36%) increased *PCSA* and force (+16%) more than *ACSA* (+10%) (Aagard et al., 2001).

Intermuscular Adipose Tissue

Many physiological/pathological conditions (e.g., aging, sedentary lifestyle, but also the augmentation of inflammatory cytokines, reduced anabolic hormonal response, and general metabolic disorders) lead to muscle deconditioning, a phenomenon characterized by a loss of muscle strength and power and an increase of fatty infiltration. Data suggest that *IMAT* may be a prominent indicator to track metabolic-dependent activity and skeletal muscle quality. The *IMAT* increase and accumulation are linked to muscle dysfunction and is largely attributable to inactivity, but it is also associated with increasing age (Marcus et al., 2010). *IMAT* negatively affects muscle quality and muscle function by decreasing absolute and specific strength levels leading to muscle weakness. The latter have been associated with high levels of *IMAT* in adults with other comorbidities (Marcus et al., 2010) and/or characterized by low levels of physical activity (Addison et al., 2014).

The efficacy of resistance training to decrease *IMAT* in adult and elderly subjects has already been documented (Nicklas et al., 2015), although the intensity of the training remains crucial: no decrease in thigh *IMAT* occurs if the eccentric intervention is carried out at submaximal intensity (Jacobs et al., 2014). Less clear and definitive data exist on the efficacy of endurance training, let alone *HIT*, to abate *IMAT* infiltration in healthy elderly subjects. The benefits of low-intensity endurance training have been evaluated (Ikenaga et al., 2017); yet, the effectiveness of *HIT* is still unknown. Notwithstanding that the present study has been conducted on a small number of subjects, the data suggest that even short periods of high-intensity aerobic training may be effective to reduce *IMAT* in elderly healthy subjects.

The improvement in the muscle quality was also highlighted by finding that the percent increases of *ACSA* obtained when *IMAT* was neglected were significantly larger than the ones obtained when *IMAT* was included in the planimetric calculation of *ACSA*.

It has been suggested that only individuals with a low infiltration of *IMAT* are able to significantly ameliorate their muscle quality (Marcus et al., 2010). In the present study, however, we observed a negative and very high correlation (r = 0.71) between the *IMAT* content before the training and the net decrease of *IMAT* observed after *HIT* and *IRT*. The regression analysis also implied that the two training modalities were able to induce an average percent decay of *IMAT* of about 25% (95% CI 51.2-1.3). These results highlight the efficacy of *HIT* and *IRT* training programs in reducing the contribution of non-functional *IMAT*.

Muscular Strength, Architecture, and Activation

Muscle strength significantly increased only after *IRT*. This type of training has been found to be effective for increasing muscular performances of the limb extensors in elderly people (Onambélé et al., 2008), and the average percent increase of T_{MVC} found in the present investigation (7.0% ± 9.8) is close to the one reported in the quoted paper. One unexpected finding consisted in the absence of any substantial and significant increase of strength after *HIT*, in spite of documented increases of *ACSA* and *Vol*. The dissociation between functional and morphological adaptations requires some extended comment.

As we know, the increase of muscular strength is a functional adaptation that results from several morphological and neural mechanisms that intervene during training (Folland and Williams, 2007). In this regard, the changes of muscular architecture are of paramount importance in order to understand how they may affect the improvement of strength consequent to the increase of muscle mass. As we have already outlined, there is a large bulk of evidence that shows that the θ_p of muscle fibers is increased in strength-trained muscles (Kawakami et al., 1993) and it augments with muscle hypertrophy (Aagard et al., 2001). This morphological change is beneficial because it will allow a greater packing of fibers for the same *ACSA* increasing *PCSA*, i.e., the area normal to the line of application of the force produced by the fibers. Of course, an increased θ_p will also decrease the force applied by the muscle along the tendon. Therefore, there is a sort of trade-off between the increase of muscle mass and the widening of θ_p on the strength measured at the ending of the tendon. Even though it has been geometrically demonstrated that any increase of θ_p that stays below 45° is compensated by the increase of *PCSA*, wherefrom an increase of strength, however, occurs, in the present case, the increase of θ_p after *HIT* was not probably sufficient to induce a substantial augmentation of *PCSA* (**Figure 5**). We must also consider that *PCSA* of the quadriceps was calculated by assuming an identical angle of θ_p measured at the level of the *VL*, which, in turn, was considered representative of the mean θ_p of the entire quadriceps. Of course, this assumption may have introduced an unpredictable error in the calculated *PCSA* of the quadriceps.

Secondly, the level of neuromuscular activation increased only after *IRT* in respect to the control condition before strength training. Even admitting that the interpolated twitch technique is not freed of several technical and methodological issues (Folland and Williams, 2007) and that activation is muscle specific (Belanger and McComas, 1981) and angle specific (Becker and Awiszus, 2001), one must acknowledge that recent studies confirm that strength training is followed by the increase of muscular activation in elderly humans (Reeves et al., 2004). Flywheel resistance training elicits a greater muscle activation of the involved muscles during isometric maximal voluntary contraction; in addition, muscular activation during both eccentric and concentric contraction seems to be maximal even in the early phases of training over the entire range of movement (Norrbrand et al., 2010). The capability of flywheel resistance training of inducing a greater neuromuscular activation may well explain the findings, after *IRT*, of a higher level of activation of the trained quadriceps. By incidence, the empirical model proposed by calculating the multiple linear regression between the percent increases of isokinetic torque and the percent increases of *ACSA* at 50% of femur length (X_1) and of %*Act* (X_2) seems to suggest that the gain in strength achieved

with *IRT* was more ascribed to the increase of muscle mass than to the amelioration of muscle activation.

HIT was not followed, though, by any substantial increase of neuromuscular activation. It has been shown that, during cycling, the four bellies of the quadriceps are activated with different timing: *VL* and *VM* show the highest activation in the pushing phase from the top dead center to about 90°, or the first quadrant of the cycle; *RF*, a bi-articular muscle, shows bursts of biphasic activation in the first (0°–90°) and fourth (270°–360°) quadrants (Lima da Silva et al., 2016). We can therefore suggest that flywheel training seems to be able to induce greater neuromuscular activation of the involved muscles along the entire range of motion in respect of cycling and, hence, contribute substantially to the increase of strength.

LIMITATIONS AND STRENGTHS

The investigation is not freed from methodological weaknesses. The primary limitation to the generalization of our results is the absence of a control group: considering the experimental design, the study was not counterbalanced. As such, it suffered from intrinsic limitations, since the results were not freed from incidental effects other than the one directly induced by the interventions, and a longer washout period between training sessions could be considered in future studies. Furthermore, the effects of training on the antagonist muscles or on all the thigh muscles could be evaluated.

In this investigation, we evaluated for the first time the effect of *HIT* and of *IRT* on the *IMAT* and muscle quality of the quadriceps in elderly subjects. In order to unveil which changes occurred in muscle tissue due to these types of trainings, a group of elderly, although healthy, untrained volunteers were investigated, since, in this category of subjects, an age-related impaired muscular response has been frequently described. Despite initial doubts about the feasibility of carrying out high-intensity workouts with elderly subjects, we found that *HIT* and *IRT*, when performed with care and customizations, are absolutely safe and well tolerated by the subjects. Therefore, the meaning and the applicability of the results obtained in this study may be relevant to address training interventions in elderly subjects: our study protocol has been applied on healthy subjects; however, the effects of *HIT* and *IRT* on *IMAT* must also be evaluated in sarcopenic subjects.

CONCLUSION

Although these results must be interpreted with caution and the limitations of the study should be borne in mind, our results indicate that *HIT* and *IRT* seem to be able to elicit beneficial modifications of skeletal muscular mass, architecture, and quality in active elderly subjects in connection with an amelioration of the functional performances (strength, power, neuromuscular activation, etc.). In particular, a significant reduction of *IMAT* was evident after the two training interventions, a fact that led to the amplification of the percent changes of *ACSA* of the muscles when they were calculated without considering *IMAT*.

IMAT is an important predictor of muscle metabolism and also appears to be a modifiable muscle risk factor: adipose tissue stored in ectopic locations, as in the muscle, is connected with impaired glucose tolerance, chronic inflammation, and increased total cholesterol (Prior et al., 2007; Durheim et al., 2008; Koster et al., 2010). Physical activity and resistance or endurance training appear to be effective countermeasures against increases in *IMAT*. The exercise protocol proposed in our study has positively influenced *IMAT*: we can therefore speculate that the exercise carried out at high intensity reduces modifiable muscle risk factors.

The two training modalities caused a homogeneous increase of *ACSA* of the quadriceps at different percentages of the total muscle length. By the same token, the percent increase of *Vol* of the different bellies of the quadriceps turned out to be homogeneous. θ_p underwent an expected increase with both training modalities with a consensual increase of *PCSA* in *IRT*. However, specific strength per unit of *PCSA* did not change and the observed increases of strength seemed to be attributed to a parallel improvement of neuromuscular activation and muscle hypertrophy only after *IRT*.

We can therefore consider that both *HIT* and, especially, *IRT* induce beneficial modification on different systems with the final effect to counteract most of the causes of the morphological and functional consequences of sarcopenia.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The protocol and the methods of the study were approved by the Regional Review Board (approval on June 18, 2013) and written informed consent was obtained from each subject before entering the study.

AUTHOR CONTRIBUTIONS

PB, ET, and CC conceived the study. PB, ET, and EC collected the data. PB, ET, EC, and AR analyzed the data. PB and CC wrote the first draft of the manuscript. All authors approved final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.01260/full#supplementary-material

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Simplified Triceps Surae Muscle Volume Assessment in Older Adults

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Triceps surae (TS) muscle volume can be estimated in young adults by only considering the maximal anatomical cross-sectional area ($ACSA_{max}$) and the length of the muscle due to the presence of a constant muscle-specific shape factor. This study aimed to investigate if this simplified muscle volume assessment is also applicable in older adults or if muscle-specific shape changes with aging. MRI sequences were taken from the dominant leg of 21 older female adults. The boundaries of all three TS muscles (SOL, soleus; GM, gastrocnemius medialis; GL, gastrocnemius lateralis) were manually outlined in transverse image sequences, and muscle volume for each muscle was calculated as the integral of the obtained cross-sectional areas of the contours along the whole length of the muscle (measured volume) and, in addition, by using the average muscle-specific shape factors of each muscle obtained from the ratio of the measured volume and the product of $ACSA_{max}$ and the muscle length (estimated volume). There were no differences in the measured and estimated muscle volumes (SOL: 357.7 ± 61.8 vs. 358.8 ± 65.3 cm^3; GM: 179.5 ± 32.8 vs. 179.8 ± 33.3 cm^3; GL: 90.2 ± 15.9 vs. 90.4 ± 14.8 cm^3). However, when using the reported shape factors of younger adults instead, we found a significant ($p < 0.05$) overestimation of muscle volume for SOL and GM with average RMS differences of 6.1 and 7.6%, respectively. These results indicate that corrections of muscle-specific shape factors are needed when using the previously proposed simplified muscle volume assessment as aging may not only be accompanied with muscle atrophy but also changes in the shape of skeletal muscle.

Keywords: magnetic resonance imaging, aged, muscle reconstruction, muscle volume, shape factor

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INTRODUCTION

Muscle volume reportedly undergoes tremendous changes with maturation (O'Brien et al., 2010), several pathologies (Zoabli et al., 2008; Ji et al., 2013), mechanical loading (Folland and Williams, 2007), immobilization (Oates et al., 2010), or aging (Morse et al., 2005a). This is of functional relevance as muscle volume is an important determinant of the muscle mechanical power (O'Brien et al., 2009) and hence physical performance (Chelly and Denis, 2001). Another relevant component of physical performance is the maximum force generating capacity of a muscle, which is mainly determined by the physiological cross-sectional area (PCSA; Haxton, 1944; Aagaard et al., 2001; Fukunaga et al., 2001). In pennate muscles, it is not possible to measure

the cross-sectional area PCSA *in vivo*; however, the indirect calculation by dividing the muscle volume by fascicle length (Powell et al., 1984; Lieber and Fridén, 2000) is well accepted, yet also reliant on muscle volume assessment. Thus, muscle volume is a crucial measure for investigating the mechanisms behind the physical capacity in different populations and evaluating the effectiveness of different interventions to enhance muscle function related to changes in muscle morphology.

The assessment of muscle volume usually involves the segmentation of the muscle from magnetic resonance imaging (MRI) recordings, which is a time-consuming procedure and, therefore, often limited for the application in clinical or research settings. Regarding this issue, Albracht et al. (2008) introduced a simplified method for the assessment of human triceps surae (TS) muscle volume *in vivo*. The approach is based on the assumption that muscle volume can be calculated as the product of the muscle length and average anatomical cross-sectional area (ACSA) with the latter being a constant muscle shape-dependent fraction (i.e., shape factor) of the maximal ACSA (Albracht et al., 2008). Assuming the muscle-specific shape to be relatively constant across populations, TS muscle volume calculation only requires the determination of the relatively easy assessable individual maximum ACSA and muscle length. Indeed, in the study of Mersmann et al. (2014), TS shape factors of young untrained, endurance, and strength trained adults were found to be in a good agreement, despite large differences in corresponding muscle volumes. Hence, gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and soleus (SOL) muscle volume of an independent group of recreationally active subjects were precisely estimated using the corresponding average shape factors from the mentioned three groups. However, in order to provide a prospect for the application of the reported time-saving assessment method in scientific and clinical settings, it is further necessary to determine whether the TS muscle shape features a similar consistency across different age populations. As there is evidence that aging leads to significant changes in muscle architecture (e.g., Narici et al., 2003) and affects the muscle volume distribution at the lower limbs (e.g., Thom et al., 2005), it seems reasonable to suggest that there might be differences in muscle shape between young and older adults. Moreover, there is proof for an inhomogeneous TS muscle atrophy along the length of the muscle due to immobilization (Akima et al., 2000; Miokovic et al., 2012), which would affect the ratio of average to maximum ACSA (i.e., shape factor). Accordingly, the generalizability of the reported TS shape factors of young adults' muscles to muscles that underwent atrophy due to aging cannot be assumed *a priori* and needs to be verified.

Therefore, the aim of the present study was to investigate if the previously proposed simplified muscle volume assessment method (i.e., shape factor-based assessment) for the TS muscle group (i.e., SOL, GM, and GL) is also valid for older adults. As a second step, we aimed to cross-validate the shape factor reported by Albracht et al. (2008) with the TS morphology data determined from the current group of older adults to extend the examination of TS muscle shape consistency across different age groups. We hypothesized that the assessment of muscle volume using the maximum ACSA and muscle length in older adults and the shape factors provided by the literature from young adults (Albracht et al., 2008) would provide discrepancies in the assessment of muscle volume, and an age-specific correction of the shape factors is required.

MATERIALS AND METHODS

Participants

The study was conducted with 21 healthy older female adults aged between 60 and 75 years [age: 65 ± 7 years; body mass: 63 ± 9 kg; body height: 165 ± 5 cm; mean; and standard deviation (SD)] who agreed to have their dominant limb scanned using MRI. Exclusion criteria were any musculoskeletal or neurological impairments of the lower limbs or pain during daily life within the last 2 years, which might influence the findings of the current study. The participating older adults were recreationally physically active and representative for their age group (mean outcome of the SF-36 general health questionnaire of 69.9%; average single leg stance time of 43.0 s out of maximal 45 s test duration; mean timed up and go test result of 7.1 s). The study was approved by the responsible ethics committees (German Sport University Cologne), and all participants provided their written informed consent after being informed about the procedures and possible risks.

Data Acquisition

Image sequences of the lower limb were acquired with a 3 Tesla MRI scanner in transverse and sagittal plane between the femur condyles and the calcaneal tuberosity in an unloaded supine position with the hip and knee fully extended and the ankle joint fixed at 20° plantar flexion (TS muscle-tendon unit close to slack position; De Monte et al., 2006). The sequences were acquired with a slice thickness of 1.0 mm, no inter-slice spacing. The sagittal sequences were recorded for the later analysis of the anatomical landmarks (origin and insertion) of all three TS muscles (i.e., SOL, GM, and GL; see **Figure 1**).

To measure the volume of the TS, the boundaries of individual muscles (SOL, GM, and GL) were manually outlined in every second transverse image at every 2 mm along the whole muscle length between the two marginal slices using a custom routine of the image processing program ImageJ (ImageJ 1.48v; National Institutes of Health, USA). In order to prevent an overestimation of TS, muscle mass, subcutaneous fat, vessels, tendon, and aponeuroses were excluded in the segmentation process (Fukunaga et al., 1992). The acquired coordinates and contours were exported as 3D coordinates and further processed using custom routines in MATLAB 2018a (The MathWorks, Natick, MA, USA; see **Figure 1**). The resulting contours were used to calculate the muscle volume ($V_{measured}$) as the integral of the obtained ACSA of the contours along the whole length of the muscle (L_{muscle}), which was determined as the distance between the two marginal slices contributing to muscle reconstruction along the longitudinal axis of the coordinate system (distance between the obtained transverse images).

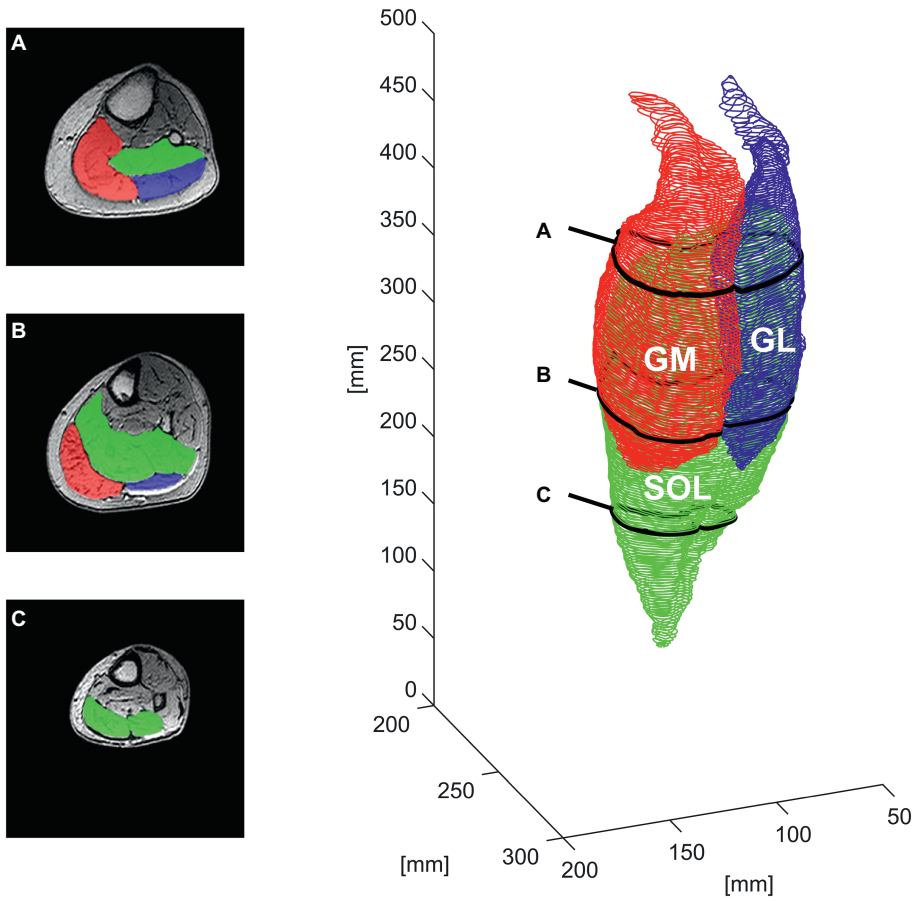

FIGURE 1 | Manual segmentation of the contours of the gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and soleus muscle (SOL) for one representative older adult in the transverse plane at ~85% **(A)**, ~60% **(B)**, and ~30% **(C)** of the shank length (left) and the respective whole-muscle reconstruction (right).

Investigation of Muscle-Specific Shape and Muscle Volume Estimation

Based on the proposed theoretical consideration of Albracht et al. (2008) that $V_{measured}$ is the product of its mean ACSA and L_{muscle}, according to which the mean ACSA may be described as a fraction (p; i.e., shape factor) of the maximum ACSA ($ACSA_{max}$), the individual TS shape factors were obtained for older adults from the assessed muscle reconstructions by dividing the measured muscle volume by the product of $ACSA_{max}$ and the L_{muscle}:

$$p = \frac{V_{measured}}{ACSA_{max} \times L_{muscle}} \quad (1)$$

In order to evaluate the applicability of the muscle volume assessment ($V_{estimated}$) based on the newly calculated muscle shape, the $V_{measured}$ obtained by whole-muscle segmentation was compared to those predicted based on Eq. (2) using the measured $ACSA_{max}$ and the L_{muscle}, and the average shape factor obtained from the whole group of analyzed older adults.

$$V_{estimated} = p \times ACSA_{max} \times L_{muscle} \quad (2)$$

In addition, we aimed to examine whether the previously reported muscle shape factors for younger subjects (Albracht et al., 2008) are valid for muscle volume assessment in the elderly. We therefore cross-validated the previously reported shape factor and compared the measured volumes to volumes estimated from Eq. (2) using measured $ACSA_{max}$ and L_{muscle} values from the present data set of older adults and average shape factors (for each investigated muscle) calculated from the group of healthy younger adults reported by Albracht et al. (2008); n = 13; age: 29 ± 6 years; body mass: 76 ± 6 kg; body height: 180 ± 4 cm; average shape factors: 0.496, 0.592, and 0.569 for SOL, GM, and GL, respectively.

Statistics

A one-way analysis of variance (ANOVA) with investigated muscle (i.e., SOL, GM, and GL) as factor was used to detect potential differences in the analyzed muscle morphological characteristics [muscle specific shape factor (p), $V_{measured}$, $ASCA_{max}$, position of the maximum ACSA relative to the shank length, and L_{muscle}] between the three components of the TS in our group of older adults. A Bonferroni *post hoc* test was applied

to identify potential differences between the three compartments of the TS regarding the muscle-specific shape factor p, $V_{measured,}$ $ASCA_{max}$, the position of the maximum ACSA relative to the shank length, and L_{muscle}. Inter-subject variability of shape factors and the position of the maximal ACSA relative to the shank were calculated using the coefficient of variance.

For the validation of the muscle volume assessment based on the muscle shape, the estimated muscle volume (i.e., SOL, GM, and GL) using the shape factors obtained from the current pool of older adults and the one measured from the whole muscle MRI analysis was compared by means of a paired sample t test after checking for normal distribution using the Kolmogorov-Smirnov test. For accuracy evaluation, the root mean squares (RMSs) of the differences between estimated and measured volume as well as the coefficient of determination (R^2) were calculated. To further test the validity of the shape factor-based assessment and whether the TS muscle shape factors feature a similar consistency across age populations, we cross-validated the muscle shape factors of younger subjects reported by Albracht et al. (2008) with the determined TS volume using the same statistical procedure as implemented using the muscle shape factors of older adults. The level of statistical significance was set at $\alpha = 0.05$, and all results in the text, tables, and figures are presented as mean and SD. All statistical analyses were performed using Statistica software (release 10.0; Statsoft, Tulsa, OK, USA).

RESULTS

Triceps Surae Muscle Morphology

A significant ($p < 0.01$) muscle effect was found for all analyzed morphological parameters within the TS (muscle specific shape factor p, $V_{measured}$, $ACSA_{max}$, position of the maximum ACSA relative to the shank, and L_{muscle}) with the SOL showing the largest muscle length, $ACSA_{max}$, and volume, followed by the GM and GL (**Table 1, Figure 2**). Muscle-specific shape factor displayed significantly ($p < 0.05$) smaller values for SOL (0.484 ± 0.027) in comparison to GM (0.556 ± 0.028) and GL (0.568 ± 0.049), whereas no differences between the two gastrocnemii muscles were detected. The inter-subject variability of the shape factors, described by the coefficient of variation, showed low values for all analyzed muscles with 5.6, 5.0, and 8.6% for SOL, GM, and GL, respectively. Furthermore, the maximum ACSA was located at 59.3 ± 3.5, 75.0 ± 4.3, and 80.6 ± 5.1% of the shank length (measured from tuberositas calcanei to the tibial plateau) for the SOL, GM, and GL, respectively (significantly different locations between all muscles; see **Figure 2**), and the inter-subject variability (coefficient of variance) of the $ACSA_{max}$ location ranged between 5.8 and 6.4%.

Triceps Surae Muscle Volume Assessment

There were no significant differences between the muscle volumes obtained from whole-muscle segmentation and the volumes predicted using the measured $ACSA_{max}$, muscle length, and the average shape factors obtained from the current pool of older adults (**Table 1, Figure 3**). Furthermore, the coefficient of determination (R^2) for the assessed muscle volumes using the newly calculated shape factors from older adults was quite high for all three muscles (0.90, 0.92, and 0.74, respectively, for SOL, GM, and GL). The relative RMS difference between the measured and estimated muscle volumes using the newly calculated shape factors from the current older adults was 4.9, 4.5, and 7.9% for SOL, GM, and GL, respectively (**Figure 3**).

When estimating muscle volume by using the shape factors previously reported by Albracht et al. (2008) for younger adults (0.496, 0.592, and 0.569 for SOL, GM, and GL, respectively), a significant ($p < 0.05$) overestimation of the muscle volume was detected for SOL and GM compartments of the TS compared to the volumes measured from whole-muscle reconstruction (with relative RMS differences of 6.1% for SOL and 7.6% for GM; **Table 1, Figures 3, 4**). No significant differences between the measured muscle volume and either of the estimated muscle volumes were detected for the GL and relative RMS differences of 7.9% (**Table 1, Figures 3, 4**). Because the individual values of $ACSA_{max}$ and L_{muscle} were the same for both estimation approaches, the coefficients of determination did not differ as well (**Figures 3, 4**).

DISCUSSION

Previous studies reported that it is possible to assess the individual muscle volume within the TS muscle group in younger adults by only using the maximal ACSA, the length of the muscle, and a muscle-specific shape factor (Albracht et al., 2008; Mersmann et al., 2014). However, whether this simplified method is also valid for muscles that underwent

TABLE 1 | Means ± standard deviations of the maximal anatomical cross-sectional area ($ACSA_{max}$), measured muscle volume ($V_{measured}$) and estimated muscle volume with shape factor from current older adults ($V_{estimated\ OLD}$) and young adults ($V_{estimated\ YOUNG}$) of the soleus (SOL), gastrocnemius medialis (GM) and gastrocnemius lateralis muscles (GL).

Muscle	L_{muscle} (cm)	$ACSA_{max}$ (cm^2)	$V_{measured}$ (cm^3)	$V_{estimated\ OLD}$ (cm^3)	$V_{estimated\ YOUNG}$ (cm^3)
SOL	30.8 ± 0.7	24.0 ± 4.4	357.7 ± 61.8	358.8 ± 65.3	367.7 ± 66.9[a]
GM	24.7 ± 1.4[*]	13.1 ± 2.3[*]	179.5 ± 32.8[*]	179.8 ± 33.3	191.4 ± 35.5[a]
GL	21.2 ± 2.1[*,#]	7.6 ± 1.2[*,#]	90.2 ± 15.9[*,#]	90.4 ± 14.8	90.5 ± 14.9

[*] *Statistically significant ($p < 0.05$) differences to SOL.*
[#] *Statistically significant ($p < 0.05$) differences to GM.*
[a] *Statistically significant ($p < 0.05$) differences to $V_{measured}$.*

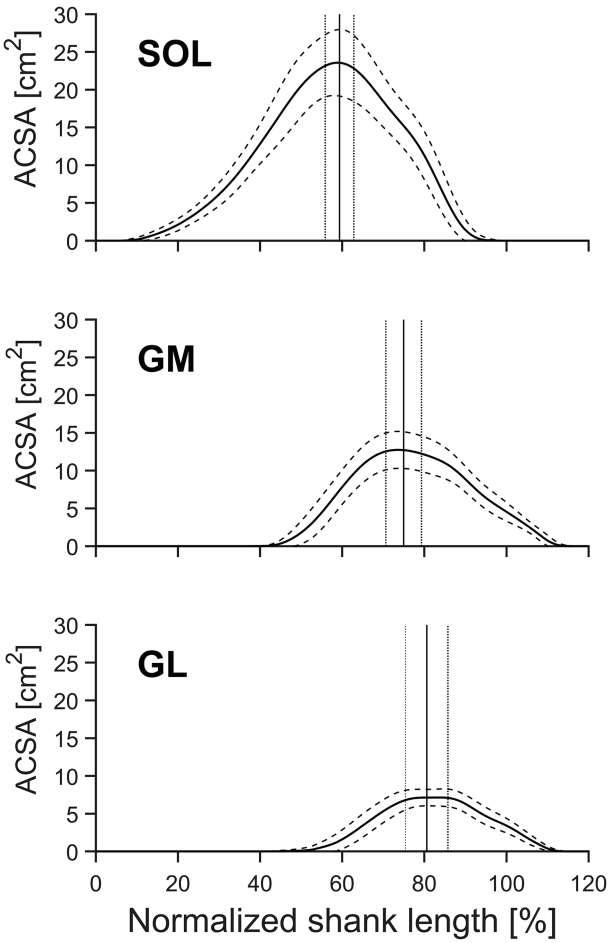

FIGURE 2 | Mean anatomical cross-sectional area (ACSA) and standard deviation (SD) of the gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and soleus muscle (SOL) of older adults (n = 21) as a function of relative shank length [from distal (0%) to proximal (100%)]. The vertical lines indicate the mean position ± SD (dotted lines) of the maximum ACSA of the pooled data. Solid lines indicate the means, and dashed lines indicate the SD.

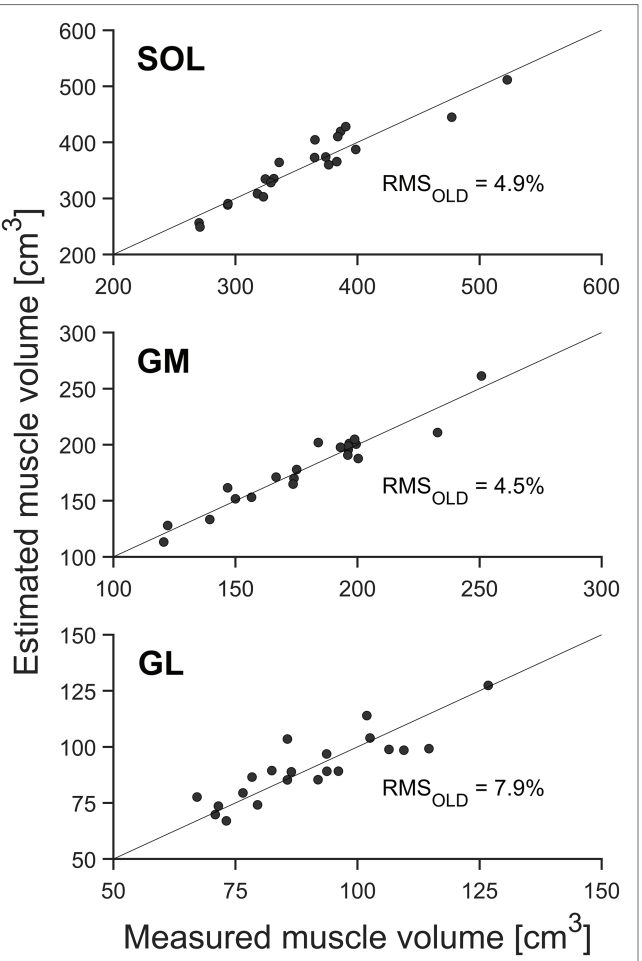

FIGURE 3 | Muscle volume of the gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and soleus muscle (SOL; n = 21) by means of whole-muscle segmentation (abscissa) in relation to estimated muscle volume using the muscle-specific shape factors of older adults (ordinate). The solid diagonal line represents the identity line. The relative root mean square (RMS) of the differences between methods is included in the figure.

atrophy due to aging is not established. The current findings suggest that the previously proposed simplified approach for assessing muscle volume by using shape factors is applicable for scientific or clinical use in the older population. However, the results also indicate that using the muscle-specific shape factor from younger adults partly overestimates the muscle volume in the TS muscles in the elderly, providing evidence that aging may not only be accompanied with muscle atrophy but also by changes in muscle shape.

Albracht et al. (2008) reported that the size of the ratio between muscle volume and the product of maximal ACSA and length of the muscle (i.e., the shape factor p) is muscle specific and hence not consistent within the TS muscle in young adults. In accordance with these findings, the data of our older population revealed that the value of p was significantly different between the SOL (0.484 ± 0.027) and both gastrocnemii muscles (GM: 0.556 ± 0.028; GL: 0.568 ± 0.049), whereas no significant difference was detected between the GM and GL (indicating a similar ratio of average and maximum ACSA). However, despite these differences within the TS, we found a considerably low inter-subject variability for p (coefficient of variance between 5.0 and 8.6%), thus indicating that the shape of each muscle seems to be similar across the examined population of older adults. Due to the markedly low inter-subject variability of the shape factor p, the assessment of muscle volume from $ACSA_{max}$ and muscle length was possible with a generally good agreement (coefficients of determination for the assessed muscle volume were quite high) and relative RMS differences between 4.5 and 7.9%. These error of prediction and coefficient of determination values within our group of older adults are in accordance with the values reported in younger adults (Albracht et al., 2008), showing that the previously proposed simplified muscle volume assessment for the TS muscle group is applicable for older adults.

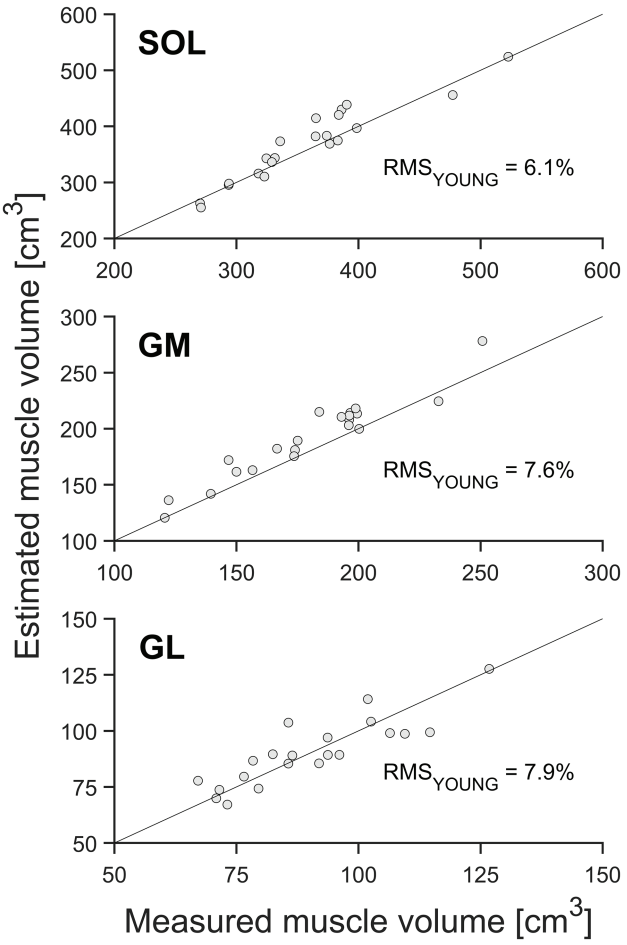

FIGURE 4 | Muscle volume of the gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and soleus muscle (SOL; $n = 21$) by means of whole-muscle segmentation (abscissa) in relation to estimated muscle volume using the muscle-specific shape factors of the young adults (ordinate). The solid diagonal line represents the identity line. The relative root mean square (RMS) of the differences between methods is included in the figure.

It is important to note, however, that the relative RMS difference of 7.9% between measured and estimated GL muscle volume was clearly higher than the corresponding value for the SOL (4.9%) or GM (4.5%) muscle. Higher error of prediction for the GL in comparison with SOL or GM has also been previously reported in young adults (Albracht et al., 2008), indicating that the error of prediction is muscle but not age dependent. The absolute volume of the GL is clearly lowest (e.g., approximately a quarter of the size of the SOL), and we argue that the GL is therefore more vulnerable to occurring errors in the manual muscle ACSA segmentation leading to higher relative errors. Nevertheless, the previously reported values of relative volume changes following strength training or sarcopenia have been considerably larger for all TS muscles (Morse et al., 2005a,b). For example, aging has been found to be associated with decreases in TS muscle volume of 17–29% (Thom et al., 2005; Morse et al., 2005a). Further, Morse et al. (2005b) reported an average increase in 11, 15, and 19% for SOL, GM, and GL muscle volume, respectively, following a 12-month resistance training program in older adults. Based on the above results, we can conclude that a simplified assessment method using the muscle shape factor, the muscle length, and its maximum ACSA for muscle volume estimation is sensitive enough to detect exercise-related hypertrophic responses of the plantar flexors as well as muscle atrophy-related changes induced by aging.

In contrast to the above findings, when using the shape factors reported in the literature from younger adults (Albracht et al., 2008), we found a significant overestimation of the predicted muscle volume for SOL and GM compared to the values measured based on the whole-muscle reconstruction. Further, the prediction errors for the GM (7.6%) and SOL (6.1%) muscle volume were larger using shape factors from young adults compared to those using the average shape factor from the older adults (4.9 and 4.5%, respectively). Thus, the assessment of muscle volume using the maximum ACSA, muscle length in older adults, and the shape factors provided by the literature from young adults (Albracht et al., 2008) provides less accurate results (at least for SOL and GM) than using the newly obtained shape factor, indicating that muscle-specific shape factors may change with aging. Accordingly, the generalizability of the reported TS shape factors of young adults' muscles to atrophied muscles of older adults could not be verified, and TS muscle shape consistency across different age groups is not given. This suggestion is further supported by the fact that the inter-subject variability (coefficient of variance) of SOL (5.6%) and GM (5.0%) muscle shape factors within our group of older adults was considerably small and is in accordance with the inter-subject variability values previously reported in young adults (Albracht et al., 2008). This means that the change in muscle shape suggested by our results seems to be a quite consistent age-related development.

Alterations in muscle shape might occur due to an inhomogeneous muscle atrophy along the length of the muscles, which has been previously reported for the TS muscle following several weeks of immobilization (Akima et al., 2000; Miokovic et al., 2012). For instance, Miokovic et al. (2012) demonstrated that atrophy of the GM and GL was greatest in their distal parts, which may lead to lower shape factors. Further, when considering all plantar flexor muscles as a whole, there was a tendency toward distal portions of the TS to be more affected during bed rest with no-exercise (Akima et al., 2000). Although it remains questionable whether such unloading paradigms appropriately represent the process of age-related atrophy (in terms of non-uniformity), these results together with the current findings suggest that aging may not only be accompanied with general loss of muscle mass but also with changes in the shape of skeletal muscle. Regarding this issue, various studies have shown that human muscles are divided into architectural subregions (Segal et al., 1991) and demonstrate region-specific differences in fiber type distribution

(Johnson et al., 1973; Elder et al., 1982) as well as distinct innervation patterns (Yang et al., 1998; Buckland et al., 2009). Hence, during human movement, these different muscle subdivisions may have slightly different functions. As older than young adults show altered locomotion mechanics and ankle joint kinetics (Karamanidis and Arampatzis, 2007), it seems possible that specific regions of the GM and SOL muscle are recruited differently causing an inhomogeneous TS muscle atrophy in response to aging.

It is important to note that in the current investigation on a group of older adults, the position of the maximum ACSA was at 59.3 ± 3.5, 75.0 ± 4.3, and 80.6 ± 5.1% of the shank length for SOL, GM, and GL, respectively, and showed low inter-individual variability (range between 5.8 and 6.4%). Due to the unimodal distribution of the muscle ACSA, the $ACSA_{max}$ can easily be identified with a few segmentations at the approximated positions (see **Figure 2**). Therefore, calculating the muscle volume using the proposed method and shape factors for older adults greatly reduces the required time for muscle volume assessment compared with full muscle segmentation.

A limitation of the current study may be the relatively low number of participants (n = 21), which reduces the potential for detecting statistical differences between methods. Due to the fact that the inter-subject variation in shape factors and the location of the maximum ACSA relative to the shank were very low for the analyzed muscles (coefficient of variation was on average less than 6%), we do not think that increasing the number of subjects would lead to meaningful alterations to our main conclusions. Regarding this, we wish to point out that there was a statistically significant overestimation for GM and SO muscle volumes when using the average shape factor from the young but not from the older adults. Although we found this overestimation of muscle volumes for older adults when using average muscle shape factors from young adults, the RMS differences were rather low. However, it is important to note that the participants in the current study were on average 65 years of age; hence, it seems possible that such differences may be even more pronounced in frail older adults with more advanced age-related atrophy. Combining the current results with those from our previous investigations conducted in young adults (Albracht et al., 2008; Mersmann et al., 2014), we propose that the simplified method is valid for assessment of muscle volume in clinical and research settings across the adult lifespan (**Figure 5**).

In conclusion, the results of the present study demonstrate that the previously proposed simplified method for assessing TS muscle volume by using the maximal cross-sectional area, muscle length, and a muscle-specific shape factor is applicable for scientific and clinical use in the older population. However, we found evidence for age-specific shape factors and hence limited generalizability among different subject groups, indicating that aging may not only be accompanied

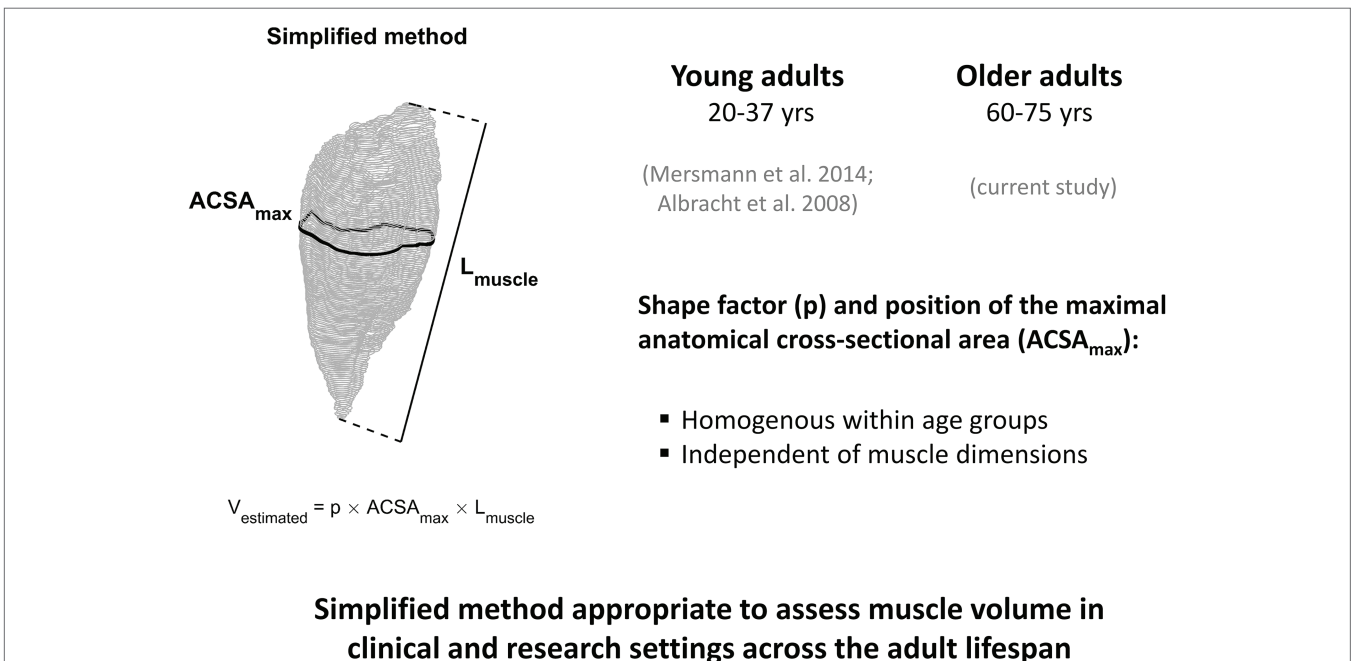

FIGURE 5 | An illustration summarizing the results of the current study in older adults and those from our previous investigations conducted in young adults (Albracht et al., 2008; Mersmann et al., 2014). Based on the observation that the position of the maximum ACSA as well as the muscle specific shape factor does not depend on muscle dimensions and is homogeneous within age groups, we propose that the simplified method is valid for assessment of muscle volume across the adult lifespan. Implementation of this method can greatly reduce the time required for muscle volume assessment compared with full muscle segmentation, an important consideration for clinical and research settings, especially in large cohort studies.

by overall muscle atrophy but also a change in the shape of skeletal muscles.

DATA AVAILABILITY STATEMENT

The data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee of the German Sport University Cologne. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KK and AA contributed to conception of the work. KK and GE performed the data acquisition. KK drafted the manuscript. KK and GE prepared the figures. All authors contributed to analysis and interpretation, approved the final version of the manuscript, and agreed to be accountable for the work.

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Triceps Surae Muscle Architecture Adaptations to Eccentric Training

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Background: Eccentric exercises have been used in physical training, injury prevention, and rehabilitation programs. The systematic use of eccentric training promotes specific morphological adaptations on skeletal muscles. However, synergistic muscles, such as the triceps surae components, might display different structural adaptations due to differences in architecture, function, and load sharing. Therefore, the purpose of this study was to determine the effects of an eccentric training program on the triceps surae (GM, gastrocnemius medialis; GL, gastrocnemius lateralis; and SO, soleus) muscle architecture.

Methods: Twenty healthy male subjects (26 ± 4 years) underwent a 4-week control period followed by a 12-week eccentric training program. Muscle architecture [fascicle length (FL), pennation angle (PA), and muscle thickness (MT)] of GM, GL, and SO was evaluated every 4 weeks by ultrasonography.

Results: Fascicle lengths (GM: 13.2%; GL: 8.8%; SO: 21%) and MT (GM: 14.9%; GL: 15.3%; SO: 19.1%) increased from pre- to post-training, whereas PAs remained similar. GM and SO FL and MT increased up to the 8th training week, whereas GL FL increased up to the 4th week. SO displayed the highest, and GL the smallest gains in FL post-training.

Conclusion: All three synergistic plantar flexor muscles increased FL and MT with eccentric training. MT increased similarly among the synergistic muscles, while the muscle with the shortest FL at baseline (SO) showed the greatest increase in FL.

Keywords: eccentric exercise, muscle architecture, muscle plasticity, triceps surae, ultrasonography

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INTRODUCTION

Muscle architecture (i.e., the geometrical arrangement by which muscle fibers are organized with respect to a muscle's line of action) has an important role in skeletal muscle force production (Fukunaga et al., 1997; Lieber and Fridén, 2000). Fascicle length (FL) is associated with the serial sarcomere number, which has a direct impact on the fiber/muscle shortening velocity and excursion (Edman et al., 1985; Lieber and Fridén, 2000; Blazevich, 2006). Longer FL is associated with

improved performance in activities demanding high velocities of shortening (Abe et al., 2000, 2001; Kumagai et al., 2000), whereas a reduction in FL with aging explains a large part of the reduced maximal shortening velocity in older compared to young adults (Thom et al., 2007). Muscle fiber pennation angle (PA) is related to the parallel number of sarcomeres within a fiber, and is related to the fiber diameter and its maximal capacity for force production (Fukunaga et al., 1997; Blazevich and Sharp, 2005). Hypertrophied muscles (i.e., with a large number of sarcomeres in parallel) tend to have high PA (Kawakami et al., 1993), while muscles of elderly (Narici et al., 2003) or disuse atrophied muscles (Suetta et al., 2009) (i.e., with a small number of parallel sarcomeres), tend to have small PA. Muscle thickness (MT) is influenced by both the serial and parallel sarcomere number, as it depends on FL and PA (Fukunaga et al., 1997; Baroni et al., 2013b).

Structural changes accompanying (or due to) strength training provide insights into the muscle's ability to accommodate architecture adaptations specific to the movement's mechanical demands. Although traditional isoinertial resistance training is executed with a constant external load in the concentric and eccentric phases of movement (Walker et al., 2016), training programs often involve isometric contractions (Kitai and Sale, 1989; Oranchuk et al., 2019) or have an emphasis on the concentric (Blazevich et al., 2007; Timmins et al., 2016b) or eccentric (Baroni et al., 2013b; Geremia et al., 2018b) phases. Evidence suggests that training programs neglecting eccentric actions do not prepare subjects for the eccentric demands encountered in sports and activities of daily living (Barstow et al., 2003; LaStayo et al., 2003; Lovering and Brooks, 2014; Franchi et al., 2017). Therefore, strength training with an emphasis on eccentric movement execution has become popular for the purpose of injury prevention (LaStayo et al., 2003; Goode et al., 2015) and rehabilitation (Rees et al., 2008; Murtaugh and Ihm, 2013; Frizziero et al., 2014), as well as physical fitness improvement in healthy subjects (Baroni et al., 2013c; Geremia et al., 2018a,b).

Several studies demonstrated changes in knee extensor muscle architecture with eccentric training (Higbie et al., 1996; Hortobágyi et al., 1996; Housh et al., 1996; Seger et al., 1998; Reeves et al., 2003; Blazevich et al., 2007; Santos et al., 2010; Raj et al., 2012; Baroni et al., 2013b,c, 2015). Most of these studies reported an increase in FL (Blazevich and Sharp, 2005; Blazevich et al., 2007; Baroni et al., 2013b), while results on changes in PA were mixed (Blazevich et al., 2007; Reeves et al., 2009; Raj et al., 2012; Baroni et al., 2013b). Eccentric training has been found to produce significant changes in muscle architecture after training periods as short as 4 weeks (Baroni et al., 2013b,c; Geremia et al., 2018b). However, different muscle groups (Mulder et al., 2009) or even muscles within the same functional group (Butterfield et al., 2005), may adapt differently when exposed to the same eccentric training stimulus.

Eccentric training programs for the triceps surae muscle have been widely used in tendon injury rehabilitation (Alfredson et al., 1998; Kingma et al., 2007; Woodley et al., 2007), as well as to improve performance in healthy subjects (Geremia et al., 2018a,b). However, findings on the effects of eccentric training on the triceps surae muscles are conflicting. While some studies show that eccentric training promotes an increase in FL, PA, and MT (Duclay et al., 2009; Geremia et al., 2018b), other studies did not find changes in these architectural outcomes (Raj et al., 2012; Fouré et al., 2013). In the majority of studies (Duclay et al., 2009; Raj et al., 2012), it was assumed that adaptations observed for the gastrocnemius medialis (GM) were representative of the entire triceps surae muscle group. However, different muscle architecture adaptations were observed after 6 weeks of stretching between the GM and the gastrocnemius lateralis (GL) (Simpson et al., 2017). This may be due to the fact that load sharing between the synergistic muscles is not homogeneous, and different mechanical loads have been observed for the different components of the triceps surae muscle (Crouzier et al., 2018). Also, differential adaptations to eccentric training have been observed for the vastus lateralis and vastus intermedius of rats exposed to a chronic downhill training protocol (Butterfield et al., 2005). Furthermore, short muscle fibers are more susceptible to muscle damage caused by eccentric training than long muscle fibers (Proske and Morgan, 2001; Baroni et al., 2013b). Considering the differences in muscle fascicular geometry of the triceps surae components (GM, GL, and SO, soleus) (Wickiewicz et al., 1983; Maganaris et al., 1998; Fouré et al., 2013), eccentric exercise may cause different levels of micro-damage in these muscles. Therefore, the idea of a specific adaptive response for the different components of the triceps surae muscle seems reasonable.

We have previously demonstrated time-dependent architectural adaptations in human knee extensor muscles (Baroni et al., 2013b). However, we were unable to find studies evaluating time-dependent adaptations in muscle architecture for the three triceps surae muscles. Considering the conflicting results regarding eccentric training adaptations of the triceps surae muscles, the limited information on architectural adaptations of the individual triceps surae muscles, and the lack of longitudinal, time-dependent adaptations of muscles subjected to eccentric training programs, the purpose of this study was to determine the effects of a 12-week eccentric training program on the architecture of the GM, GL, and SO muscles. Based on the literature (Proske and Morgan, 2001; Duclay et al., 2009; Baroni et al., 2013b; Geremia et al., 2018b), we hypothesize that eccentric training will promote adaptations in muscle architecture, causing larger increases in FL and MT in the muscle with the shortest FL (i.e., SO). In addition, we also expected that these adaptations will occur between 4 and 8 weeks of the training program.

MATERIALS AND METHODS

Participants

All procedures in this study were approved by the Ethics Research Committee of the Universidade Federal do Rio Grande do Sul (Protocol number: 787.347; CAAE: 32907414.9.0000.5347). All participants signed an informed consent form prior to their participation. Healthy and physically active male subjects (18–35 years of age) were invited to participate in the study. Participants were excluded if (1) they were enrolled in any

lower limb strength training program within 6 months of this study; (2) they had any musculoskeletal injury of the lower and/or the upper limbs; (3) they had any contra-indications for maximal effort contractions (cardiovascular, musculoskeletal, respiratory, or neurologic diseases); (4) they had any difficulty in understanding and/or executing the testing and training protocols at the isokinetic dynamometer; or if (5) they missed two or more of the training sessions.

The G*Power software (Kiel University, Germany) was used to calculate the sample size of 15 subjects using an effect size (ES) of 0.30, a significance level of 0.05; and a power of 0.80 (Rhea, 2004; Faul et al., 2007; Baroni et al., 2013c; Geremia et al., 2018b). Twenty-four participants started the training program. One participant abandoned the study for personal reasons, and three participants were excluded due to ankle pain during the training program. Therefore, 20 participants (university students; 26 ± 4 years; 1.75 ± 0.08 m height; 75 ± 9 kg of body mass; 24 ± 2 kg/m^2 of body mass index), physically active men, completed the eccentric training program. Fourteen participants completed all 23 training sessions, and six participants completed 22 training sessions (adherence: 98.8%).

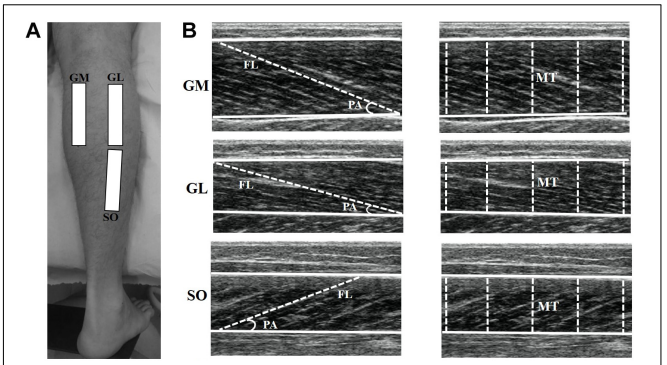

FIGURE 1 | Representation of the ultrasound sites in the gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and soleus (SO) muscles **(A)**. The ultrasonography images show GM, GL, and SO muscles **(B)**. For each muscle, superficial and deep aponeuroses are visualized (continuous lines), among which the fascicle length (FL) and muscle thickness (MT) were measured. Pennation angle (PA) was calculated as the angle between the muscle fascicle and the deep aponeurosis.

Experimental Design

A longitudinal trial was designed to determine morphological adaptations of GM, GL, and SO during the 12-week plantar flexor eccentric training program. Triceps surae muscle architecture was evaluated five times: at the start (Baseline); after a 4-week control period (Pre-training), in which participants were instructed not to change their regular physical exercise regimen; after 4 (Post-4); after 8 (Post-8); and after 12 (Post-12) weeks of eccentric training. All participants underwent a 4-week control period immediately before the training program (Baroni et al., 2013b,c; Geremia et al., 2018a,b). No training sessions were held in the evaluation weeks, and a 1-week interval was observed after the last training session and the evaluation session (Geremia et al., 2018b).

Measurements of Triceps Surae Muscle Architecture

A B-mode ultrasonography system (SSD-4000; Aloka Inc., Tokyo, Japan) with a linear-array probe operating at 32 Hz (UST-5710, 60 mm, 7.5 MHz, depth 6.0 cm, no image filter) was used to determine FL, PA, and MT of GM, GL, and SO. Triceps surae muscle architecture was determined through measurements conducted while the subject was seated with the hip flexed at 85° (0° = hip fully extended), the knee fully extended, and the ankle in the neutral position (foot surface perpendicular to the shank). All volunteers were instructed to not engage in any vigorous physical activity for 48 h before the tests (Baroni et al., 2013b).

Three ultrasound (US) images were obtained for each muscle with the subject at rest. The US probe was covered with water-soluble transmission gel and positioned longitudinally to the muscle fibers and perpendicular to the skin at 50% (SO) and 30% (GM, GL) of the distance between the popliteal crease and the lateral malleolus (**Figure 1A**; Kawakami et al., 1998; Fouré et al., 2013; Geremia et al., 2018b). Great care was taken to determine the sites where the images were obtained. The US probe was adjusted on the skin surface, parallel to the superficial and deep aponeuroses, and with clarity of the aligned hyperechoic perimysial intramuscular connective tissue (Bohm et al., 2018). Probe alignment was considered appropriate when superficial and deep aponeuroses were parallel, and several fascicles could be easily delineated without interruption across the image (Baroni et al., 2013b). Anatomical reference points, skin marks, and the ultrasonography scanning sites were mapped on a malleable plastic sheet to ensure that repeated scans were taken from the same site.

Ultrasonography images were analyzed by Image J software (straight line, line color: yellow, version 1.48v, National Institutes of Health, Bethesda, MA, United States). The best fascicle (i.e., the fascicle that could be seen in its entirety from its insertion on the deep aponeurosis into the superficial aponeurosis, or to the US probe field-of-view end) in each image was used for FL and PA analysis. FL was considered as the length of the fascicular path between superficial and deep aponeuroses (**Figure 1B**). When the ends of the fascicles were outside the US image, FL was estimated as recommended in previous studies (Finni et al., 2001; Avela et al., 2004; Abellaneda et al., 2009). PA was calculated as the angle between the muscle fascicle and the deep aponeurosis (**Figure 1B**). MT was defined as the distance between the deep and superficial aponeuroses, and was calculated through the mean value of five parallel lines drawn at right angles between the superficial and deep aponeuroses along each ultrasonography image (**Figure 1B**). Mean values were obtained from three US images for each muscle in order to determine FL and PA (i.e., analyses were based on a total of three fascicles per muscle), as well as MT (Baroni et al., 2013b; Geremia et al., 2018b). All measurements taken during the study were performed by the same investigator with extensive experience in ultrasonography, who was blinded to the identity of the participants and time-point at which each ultrasonography image was obtained.

Training Program

The eccentric training program was conducted for 12 weeks and was the same as that used in previous studies (Geremia et al., 2018a,b). It encompassed three 4-week mesocycles. The first mesocycle had seven training sessions; the second and third mesocycles had eight training sessions each. The smaller number of training sessions in the first mesocycle was chosen to avoid excessive muscle damage in the first training week (Baroni et al., 2013c). Therefore, the training program was comprised of 23 training sessions, and, except for the 1st week, all training sessions were executed twice a week, respecting a 72-h interval between consecutive sessions. Training volume was gradually augmented and consisted of 3 × 10 repetitions in weeks 1 to 4, 4 × 10 repetitions in weeks 5–8, and 5 × 10 repetitions in weeks 9–12.

Before each training session, participants performed a 10-min warm up on a cycle ergometer with a 100 W constant power output (Duclay et al., 2009; Geremia et al., 2018b). Next, a specific warm up (1 × 10 repetitions of submaximal plantarflexion and dorsiflexion concentric contractions at an angular velocity of $120°.s^{-1}$) was executed on an isokinetic dynamometer (*Biodex System 3 Pro, Biodex Medical Systems, United States*) with the subject seated with the hip flexed at 85° (0°= hip fully extended) and the knee fully extended. The eccentric training started at an ankle angle corresponding to 80% of the maximal dorsiflexion angle (DFmax) and covered a range of 50° (Geremia et al., 2018a,b). DFmax was re-evaluated passively at the isokinetic dynamometer every 4 weeks in order to re-adjust maximal ankle joint range of motion.

During training, participants were instructed to resist the dorsiflexion motion generated by the dynamometer using maximal effort contractions. Participants executed eccentric contractions only during the training program. At the end of each eccentric contraction, the participant's foot was passively moved back to the initial position for the next eccentric contraction. Participants were encouraged to execute maximal plantar flexion effort as soon as the dynamometer arm reached the initial position. All repetitions were executed continuously, with a 1-min interval between series. Both limbs were trained to avoid inter-limb muscle imbalances. However, only the dominant limb was used for data analysis (18 right limbs and two left limbs). Subjects were asked about the leg they used to kick a ball, which was considered the dominant leg (Geremia et al., 2015).

Statistics

All statistical analyses were executed in SPSS Statistics software (IBM, version 20, United States) with a 5% significance level ($\alpha \leq 0.05$). Results are presented in tables by means ± standard deviations, and in the figures by means ± standard errors.

An intra-class correlation coefficient (ICC) was used to determine test-retest reliability between Baseline and Pre-training values. Reliability was interpreted according to Landis and Koch's scale (Landis and Koch, 1977) as: <0.00 poor, 0.0–0.2 slight, 0.21–0.4 fair, 0.41–0.6 moderate, 0.61–0.8 substantial, and 0.81–1.0 almost perfect.

A repeated measures one-way ANOVA was used to determine possible training effects as a function of time (i.e., difference between the Baseline, Pre-training, Post-4, Post-8, and Post-12 evaluation time-points) for FL, PA, and MT for each muscle. Normality of the target variables was determined using the Shapiro–Wilk test. The Mauchly test and the Greenhouse–Geisser correction were applied when data sphericity was not obtained. A Bonferroni *post hoc* test was used to identify between time-point differences (Baseline, Pre-training, Post-4, Post-8, and Post-12) for each outcome. ES (Cohen's *d*) was calculated and classified as trivial ($d < 0.2$), small ($d > 0.2$), moderate ($d > 0.5$), or large ($d > 0.8$) (Cohen, 1988).

Relative changes to the Pre-training evaluation were determined for each outcome variable. A repeated measures two-way ANOVA was used for the between-muscles (GM, GL, and SO) and between-time points (Baseline, Pre-training, Post-4, Post-8, and Post-12) comparisons. When interaction between muscle and time-point was observed, a one-way ANOVA was used at each evaluation time to compare the relative change between the GM, GL and SO muscles, while a one-way repeated measures ANOVA, followed by a Bonferroni *post hoc* test was used in each muscle to compare the different evaluation times.

Responsiveness to the eccentric training (percent change from pre- to post-training) was determined by the typical error (TE) criteria (Cadore et al., 2018). The TE was calculated by the equation $TE = SD_{diff}/\sqrt{2}$, where SD_{diff} is the standard deviation of the differences between the evaluation time-points of Baseline and Pre-training. Non-responsive participants were defined as those that did not achieve an increase that was two times higher than the TE with respect to zero.

RESULTS

High scores for test-retest reliability between baseline and pre-training evaluations were obtained for all measures. An almost perfect (Landis and Koch, 1977) result was observed for all outcomes: FL (GM = 0.903; GL = 0.914; SO = 0.934), PA (GM = 0.897; GL = 0.884; SO = 0.904), and MT (GM = 0.975; GL = 0.982; SO = 0.979). Baseline and Pre-training values were similar for all outcomes ($p > 0.05$; ES < 0.2; **Table 1**).

All triceps surae muscles increased their FL in response to eccentric training (GM: $p < 0.001$, ES = 0.90; GL: $p < 0.001$, ES = 0.51; SO: $p < 0.001$, ES = 1.00; **Table 1**). The three muscles increased their FL in the first four training weeks. GM and SO continued to increase their FL from Post-4 to Post-8, while GL did not. None of the three muscles had FL changes between Post-8 and Post-12 (**Table 1**). The individual responsiveness analysis showed that 60–90% of the participants responded to eccentric training with FL increases (**Table 2**).

Pennation angle did not change along the training period for any muscle ($p > 0.05$; ES < 0.2; **Table 1**). According to the individual responsiveness analysis, 35–50% of the participants presented changes on PA in response to the eccentric training (**Table 2**).

Muscle thickness increase was consistent among the muscles assessed in this study (GM: $p < 0.001$, ES = 1.08; GL: $p < 0.001$, ES = 1.29; SO: $p < 0.001$, ES = 1.40; **Table 1**). Just as observed for FL, the three muscles increased their MT in the first four training

TABLE 1 | Fascicle length (FL), pennation angle (PA), and muscle thickness (MT) from gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and soleus (SO).

		Baseline	Pre-training	Post-4	Post-8	Post-12
GM	FL (cm)	$5.36^{a} \pm 0.65$	$5.33^{a} \pm 0.69$	$5.81^{b} \pm 0.72$	$6.00^{c} \pm 0.73$	$6.03^{c} \pm 0.85$
	PA (°)	$20.61^{a} \pm 2.27$	$20.35^{a} \pm 2.25$	$20.87^{a} \pm 2.34$	$20.92^{a} \pm 2.65$	$21.22^{a} \pm 2.70$
	MT (cm)	$1.86^{a} \pm 0.24$	$1.85^{a} \pm 0.23$	$1.98^{b} \pm 0.22$	$2.11^{c} \pm 0.24$	$2.12^{c} \pm 0.27$
GL	FL (cm)	$6.32^{a} \pm 1.00$	$6.39^{a} \pm 1.03$	$6.77^{b} \pm 1.15$	$6.89^{b} \pm 1.14$	$6.95^{b} \pm 1.18$
	PA (°)	$12.39^{a} \pm 1.32$	$12.59^{a} \pm 1.48$	$13.37^{a} \pm 2.15$	$13.34^{a} \pm 1.52$	$13.61^{a} \pm 2.08$
	MT (cm)	$1.37^{a} \pm 0.17$	$1.38^{a} \pm 0.18$	$1.53^{b} \pm 0.16$	$1.57^{b} \pm 0.15$	$1.60^{b} \pm 0.16$
SO	FL (cm)	$4.60^{a} \pm 0.85$	$4.62^{a} \pm 0.86$	$5.14^{b} \pm 1.07$	$5.43^{c} \pm 1.14$	$5.57^{c} \pm 1.04$
	PA (°)	$18.29^{a} \pm 1.80$	$18.58^{a} \pm 2.00$	$18.59^{a} \pm 3.94$	$19.39^{a} \pm 4.47$	$18.80^{a} \pm 3.60$
	MT (cm)	$1.55^{a} \pm 0.18$	$1.56^{a} \pm 0.18$	$1.74^{b} \pm 0.25$	$1.85^{c} \pm 0.28$	$1.85^{c} \pm 0.23$

All values are mean ± sd. Different letters indicate differences between moments ($p \leq 0.05$).

TABLE 2 | Individual responsiveness to eccentric training.

		Typical error	Responders *n* (%)	Non-responders *n* (%)
GM	FL	0.21	14 (70)	06 (30)
	PA	0.72	10 (50)	10 (50)
	MT	0.04	18 (90)	02 (10)
GL	FL	0.30	12 (60)	08 (40)
	PA	0.48	08 (40)	12 (60)
	MT	0.02	19 (95)	01 (05)
SO	FL	0.22	18 (90)	02 (10)
	PA	0.59	07 (35)	13 (65)
	MT	0.03	17 (85)	03 (15)

GM, gastrocnemius medialis; GL, gastrocnemius lateralis; SO, soleus; FL, fascicle length; PA, pennation angle; MT, muscle thickness.

weeks, and GM and SO continued to increase from Post-4 to Post-8. None of the three muscles had MT changes between Post-8 and Post-12 (**Table 1**). The individual responsiveness analysis shows that 85–95% of the participants responded to eccentric training with MT increases (**Table 2**).

As shown in **Figure 2**, the percent change analysis further supports the continuous increase in FL and MT up to eight training weeks, with no consistent change in the PA. SO and GM had greater FL percent changes than GL at Post-4, Post-8, and Post-12, while SO had greater values than GM at Post-12 (**Figure 2**). The three muscles had similar MT percent changes along the training program (**Figure 2**).

DISCUSSION

The purpose of this study was to determine the effects of a 12-week eccentric training program on the triceps surae FL, PA, and MT. The primary findings of this study were that: (1) eccentric training leads to an increase in FL and MT, while PA does not change, (2) FL of SO showed the greatest relative increase, and (3) 4 weeks of eccentric training are sufficient to cause architectural changes in all triceps surae muscles.

Fascicle length increase is a commonly found adaptation with eccentric training (Blazevich and Sharp, 2005; Blazevich et al., 2007; Duclay et al., 2009; Baroni et al., 2013b; Timmins et al., 2016b). Studies in animal models (Lynn et al., 1998; Butterfield et al., 2005) support the idea that eccentric training leads to serial sarcomere addition (sarcomerogenesis) and, consequently, increases in FL. This increase in serial sarcomeres might be related to eccentric contraction-induced muscle micro-damage, which activates processes of tissue repair potentially causing serial sarcomere addition in myofibrils (Proske and Morgan, 2001; Baroni et al., 2013b). This serial sarcomere addition increases FLs thereby reducing individual sarcomere lengths for a given muscle length and joint configuration. Since eccentric contraction-induced damage has been shown to critically depend on sarcomere length, increasing sarcomere number and decreasing sarcomere lengths has been thought to be a potent strategy for mitigating eccentric muscle damage (Morgan, 1990; Proske and Morgan, 2001; Brughelli and Cronin, 2007; Lieber, 2018). It has also been argued that increasing serial sarcomere number may increase a muscle's compliance (Proske and Allen, 2005), thereby minimizing the effects of unstable regions (descending phase) of the force-length relation during eccentric contractions (Proske and Allen, 2005; Brughelli and Cronin, 2007), but the theories of instability on the descending limb of the force-length relationship have largely been shown to be not tenable (Allinger et al., 1996; Rassier et al., 2003). Some characteristics of our eccentric training program may have potentiated the FL increase. Considering that elevated eccentric loads (Reeves et al., 2009) and high intensity stretching (Freitas and Mil-Homens, 2015) lead to FL increase, our elevated loads (3–5 series × 10 maximal repetitions) and the large elongation (80% DFmax) caused by our eccentric training program may have optimized conditions for large serial sarcomere additions, leading to the observed FL increase.

Fascicle length increase is associated with important functional changes, such as increased joint range of motion (Potier et al., 2009; Freitas and Mil-Homens, 2015), and a shift of the length of optimal force production toward longer muscle length (Brughelli and Cronin, 2007). We recently demonstrated, in the same participants, that the eccentric protocol used in this study was associated with an increase in DFmax, and a shift in the plantar flexor torque production optimal length toward longer lengths (Geremia et al., 2018b). Among the advantages of such a shift in optimal muscle length is a reduction in the risk for muscle strains by overstretching (Brockett et al., 2001;

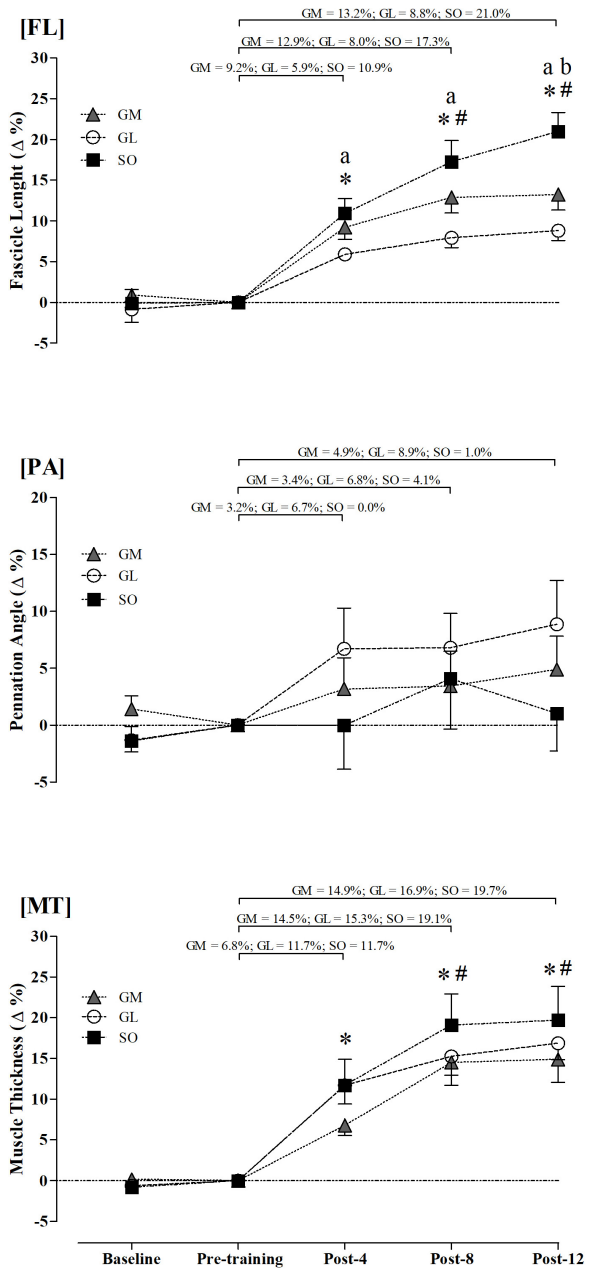

FIGURE 2 | Relative changes in fascicle length (FL), pennation angle (PA), and muscle thickness (MT) of gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and soleus (SO). *Different from pre-training ($p \leq 0.05$). #Different from Post-4 in the GM and SO muscles ($p \leq 0.05$). [a]Indicates differences between SO and GL muscles ($p \leq 0.05$). [b]Indicates differences between SO and GM muscles ($p \leq 0.05$).

Brughelli and Cronin, 2007). Plantar flexor muscle strain injuries occur frequently in sports that demand high running speed or running volumes, with high acceleration and deceleration phases (Green and Pizzari, 2017). It has also been suggested that long fascicle excursion reduces the risk for plantar flexors strain injuries (Green and Pizzari, 2017). Although there is not sufficient evidence for a causal relationship between FL and plantar flexor strain injury, such a relationship has been found for the hamstring muscles (Timmins et al., 2016a). In addition, Ribeiro-Alvares et al. (2018) demonstrated that eccentric training (Nordic hamstring exercise; 4 weeks) reduced risk factors typically associated with the hamstring strain injuries. Therefore, eccentric training might also be an important approach in reducing the risk for plantar flexor strain injuries through an increase in triceps surae FL.

An increase in FL may also have implications for sports performance. Theoretically, long-fibered muscles should be able to generate greater force at high shortening velocities compared to short-fibered muscles, as each sarcomere has a characteristic maximal speed of shortening, and this shortening speed is directly dependent on the number of sarcomeres in series in a muscle fiber (Lieber and Fridén, 2000; Blazevich, 2006). High maximal shortening velocities favor performance in which the shortening velocity is high and power requirements are large. Athletes with long FL have been shown to have better sprint performance than athletes with short fiber length (Kumagai et al., 2000; Abe et al., 2001; Mangine et al., 2014; Nasirzade et al., 2014). Kumagai et al. (2000) found a positive correlation between FL and 100 meters running performance in elite sprinters. Similarly, lower limb FL in sprint runners have been shown to be longer than in non-sprint runners (Abe et al., 2000; Lee and Piazza, 2009). Increased FL might also be an important aspect when developing training programs for the elderly. Aging is associated with neural (Clark and Manini, 2008; Aagaard et al., 2010) and structural (i.e., sarcopenia) (Andersen, 2003; Doherty, 2003) adaptations that reduce a muscle's force-velocity and power-velocity ability (Vandervoort, 2002; Baroni et al., 2013a). Sarcopenia has been associated with a reduction in FL (Doherty, 2003; Baroni et al., 2013a), and this reduction appears to account for almost half of the difference in muscle shortening velocity between young and elderly subjects (Thom et al., 2007). With that in mind, eccentric training may prove to be an important intervention to prevent sarcopenia, strength and power loss with aging (Reeves et al., 2009).

The SO showed a greater relative increase in FL than GL. This finding might be explained by the different initial FL in these muscles. Previous studies suggested that FL is a crucial determinant for eccentric training-induced muscle damage (Lieber and Friden, 1999; Proske and Morgan, 2001). Short compared to long muscle fibers are thought to be potentially more susceptible to muscle damage as they may work closer to the descending limb of the force-length relationship (Baroni et al., 2013b), causing greater strains and micro-damage in short compared to long muscle fibers (Lieber and Friden, 1999; Proske and Morgan, 2001). Among the triceps surae muscles, the SO has the shortest and GL the longest FL (Wickiewicz et al., 1983; Maganaris et al., 1998; Fouré et al., 2013). Assuming similar moment arms for these muscles, similar excursion afforded by changes in the angle of pennation, and similar compliance in the series elastic elements, this structural difference between SO and GL may lead to greater micro-damage in SO than GL, thereby explaining the greater relative increase in FL in SO compared to GL. However, in order to test this theory properly, the excursions

of the muscle fibers during the eccentric protocol would need to be quantified carefully.

In addition to an increase in serial sarcomeres, strength training is typically associated with muscle hypertrophy characterized by an increase in sarcomeres arranged in parallel (Schoenfeld, 2010), which has been used to explain increases in PA of hypertrophied muscles (Kawakami et al., 1993). Although some eccentric training studies reported an increase in PA (Blazevich et al., 2007; Duclay et al., 2009), we did not find such an increase in our study. Our results agree with previous studies (Baroni et al., 2013b; Fouré et al., 2013). The absence of a change in PA post-eccentric training might be due to measurement errors in US image analysis (Baroni et al., 2013b). While eccentric training and conventional resistance training seem to increase plantar flexor PA by around 1.2–4.0° (Morse et al., 2007; Duclay et al., 2009; Vieillevoye et al., 2010; Sanz-Lopez et al., 2016), ultrasonographic analyses have shown a TE of 0.15–3.7 (de Boer et al., 2008; Padhiar et al., 2008; Martins et al., 2012; McMahon et al., 2016). Reported PA adaptive responses to strength training are close to the measurement error in US analysis, which could explain results from studies that found an increase in muscle hypertrophy without concomitant changes in PA (Reeves et al., 2009; Raj et al., 2012; Baroni et al., 2013b; Fouré et al., 2013).

The main limitation of our study is the lack of a control group. However, a 4-week control period was used to verify the outcomes' reliability and to establish a baseline period for each participant, as previously done in other studies (Izquierdo et al., 2001; Hakkinen et al., 2003; Baroni et al., 2013b,c; Geremia et al., 2018a,b). As shown in **Table 2**, there were no changes in the outcomes during the control period. In addition, our training program used eccentric isokinetic contractions that allow for a high load and precise velocity control. However, during activities of daily living, external loads are applied with different magnitudes and at different velocities, and therefore our protocol does not resemble what happens in mechanical load exposition in everyday life. Therefore, exercises allowing a larger external load variability (e.g., use of body weight) might be more interesting for clinical practice, and it would be interesting to see if, in rehabilitation programs, the commonly used eccentric exercises do lead to similar adaptations during clinical practice as those here observed. Finally, the high intensity used in our eccentric training program can promote excessive overload on the tendon, which could contribute to the development of tendinopathy (Fredberg and Stengaard-Pedersen, 2008; Bohm et al., 2015). Therefore, although our results indicate that our eccentric training program produced important adaptations at the triceps surae muscle, care should be taken when introducing this or other high intensity eccentric exercise programs aimed at improving physical fitness, injury prevention or injury rehabilitation.

We conclude that 12 weeks of eccentric training increased the triceps surae FL and MT. However, no changes were observed for PA for any of the triceps surae muscles throughout the training program. The SO muscle presented the highest structural adaptations, whereas GL showed the smallest adaptability to eccentric training among the three synergistic muscles.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Research Committee of the Universidade Federal do Rio Grande do Sul (Protocol number: 787.347; CAAE: 32907414.9.0000.5347). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JG, BB, and MV contributed to the study conception and design. JG, FL, and AL contributed to the data acquisition. JG, BB, FL, RB, AL, WH, and MV contributed to the analysis and interpretation of data, drafting of manuscript, and critical revision. All authors approved the final version of the manuscript.

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CORRECTION
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Corrigendum: Triceps Surae Muscle Architecture Adaptations to Eccentric Training

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Keywords: eccentric exercise, muscle architecture, muscle plasticity, triceps surae, ultrasonography

A Corrigendum on

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In the original article, an incorrect abbreviation for muscle thickness, "ML," was used instead of "MT." A correction has been made to the **Abstract**, subsection **Results**:

"Fascicle lengths (GM: 13.2%; GL: 8.8%; SO: 21%) and MT (GM: 14.9%; GL: 15.3%; SO: 19.1%) increased from pre- to post-training, whereas PAs remained similar. GM and SO FL and MT increased up to the 8th training week, whereas GL FL increased up to the 4th week. SO displayed the highest, and GL the smallest gains in FL post-training."

In addition, the training responsiveness was mistakenly calculated considering the Pre-training and Post-12 moments. Thus, "Pre-training and Post-12" was published instead of "Baseline and Pre-training," which is the correct calculation. Corrections have been made in the following places:

The **Materials and Methods** section, subsection **Statistics**, the final paragraph:

"Responsiveness to the eccentric training (percent change from pre- to post-training) was determined by the typical error (TE) criteria (Cadore et al., 2018). The TE was calculated by the equation $TE = SD_{diff}/\sqrt{2}$, where SD_{diff} is the standard deviation of the differences between the evaluation time-points of Baseline and Pre-training. Non-responsive participants were defined as those that did not achieve an increase that was two times higher than the TE with respect to zero."

The **Results** section, paragraphs 2-4:

"All triceps surae muscles increased their FL in response to eccentric training (GM: $p < 0.001$, ES = 0.90; GL: $p < 0.001$, ES = 0.51; SO: $p < 0.001$, ES = 1.00; **Table 1**). The three muscles increased their FL in the first four training weeks. GM and SO continued to increase their FL from Post-4 to Post-8, while GL did not. None of the three muscles had FL changes between Post-8 and Post-12 (**Table 1**). The individual responsiveness analysis showed that 60–90% of the participants responded to eccentric training with FL increases (**Table 2**).

Pennation angle did not change along the training period for any muscle ($p > 0.05$; ES < 0.2; **Table 1**). According to the individual responsiveness analysis, 35–50% of the participants presented changes on PA in response to the eccentric training (**Table 2**).

TABLE 2 | Individual responsiveness to eccentric training.

		Typical error	Responders *n* (%)	Non-responders *n* (%)
GM	FL	0.21	14 (70)	06 (30)
	PA	0.72	10 (50)	10 (50)
	MT	0.04	18 (90)	02 (10)
GL	FL	0.30	12 (60)	08 (40)
	PA	0.48	08 (40)	12 (60)
	MT	0.02	19 (95)	01 (05)
SO	FL	0.22	18 (90)	02 (10)
	PA	0.59	07 (35)	13 (65)
	MT	0.03	17 (85)	03 (15)

GM, gastrocnemius medialis; GL, gastrocnemius lateralis; SO, soleus; FL, fascicle length; PA, pennation angle; MT, muscle thickness.

Muscle thickness increase was consistent among the muscles assessed in this study (GM: $p < 0.001$, ES = 1.08; GL: $p < 0.001$, ES = 1.29; SO: $p < 0.001$, ES = 1.40; **Table 1**). Just as observed for FL, the three muscles increased their MT in the first four training weeks, and GM and SO continued to increase from Post-4 to Post-8. None of the three muscles had MT changes between Post-8 and Post-12 (**Table 1**). The individual responsiveness analysis shows that 85–95% of the participants responded to eccentric training with MT increases (**Table 2**)."

Finally, as the training responsiveness was miscalculated, **Table 2** needs to be corrected. The corrected **Table 2** appears above.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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BRIEF RESEARCH REPORT
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Combined Aerobic and Resistance Training Increases Stretch-Shortening Cycle Potentiation and Walking Economy in Postmenopausal Women

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Purpose: Secondary analyses were performed to test whether combined aerobic and resistance training altered walking economy (i.e., net oxygen uptake) and/or stretch-shortening cycle potentiation (SSCP). A further objective was to determine if walking economy and SSCP were related before or after training.

Methods: Ninety-two postmenopausal women were enrolled wherein 76 completed 16 weeks of supervised aerobic and resistance training. Participants were randomized to one of three training groups based on frequencies: (a) 1 d · wk^{-1} (n = 23); (b) 2 d · wk^{-1} (n = 30) or; (c) 3 d · wk^{-1} (n = 23). Following assessments were performed at baseline and post-training. Indirect calorimetry was used to measure maximal oxygen uptake ($\dot{V}O_{2max}$) and walking economy (submaximal $\dot{V}O_2$ – resting $\dot{V}O_2$ = net $\dot{V}O_2$) during a graded exercise test and steady-state treadmill task, respectively. SSCP was determined by measuring the difference between a concentric (CO) and counter-movement (CM) leg press throw.

Results: $\dot{V}O_{2max}$, walking economy, CO and CM velocity were significantly improved ($p < 0.05$) for all training groups, however; no time by group interactions were observed. Paired t-tests revealed participants exercise training 2 d · wk^{-1} exhibited a significant time effect for SSCP (+0.04 ± 0.09 ms^{-1}; p = 0.03). At baseline, multiple linear regression showed a negative relationship between walking net $\dot{V}O_2$ and SSCP (r = −0.22; $p < 0.04$) adjusted for relative proportion of $\dot{V}O_{2max}$. No such relationship was found post-training.

Conclusion: Among older postmenopausal women, our results indicate that irrespective of frequency of training, 16 weeks of combined aerobic and resistance exercise training increased ease of walking and economy. Additionally, only participants exercising 2 d · wk^{-1} exhibited significant improvement in SSCP.

Keywords: exercise, walking, elastic energy, muscle contraction, potentiation, postmenopausal

INTRODUCTION

Obesity remains a major public health concern in the United States (World Health Organization, 2016). Given that habitual free-living physical activity (PA) is associated with favorable weight management (Schoeller et al., 1997; Weinsier et al., 2002), it is of interest to identify factors that may be influential to long-term PA adherence. Previously, we have shown that improved ease of walking (i.e., ↓heart rate for a given workload) increases spontaneous engagement in PA among older adults (Hunter et al., 2004a,b, 2005; Hartman et al., 2007), whereas improved walking economy, inverse of net walking $\dot{V}O_2$ facilitates overall locomotion (Larew et al., 2003; Hunter et al., 2004a,b). Indeed, modifiable lifestyle factors like exercise training represent one of the most effective strategies to combat the loss of physiologic function with advancing age.

Resistance training, in particular, has been shown to improve ease of walking and economy, both of which, appear to enhance activity energy expenditure independent of exercise training (Hunter et al., 2000, 2001, 2013). As such, it is reasonable that walking/running economy may not only be a construct in athletic performance, but also a significant factor linked to non-exercise training activity thermogenesis (NEAT) - a key feature known to mitigate weight gain in older adults (Hunter et al., 2001, 2004b, 2013; Weinsier et al., 2002; Hunter and Byrne, 2005). Thus, a clearer understanding of factors that govern walking economy could be used to inform efficient exercise strategies to facilitate greater PA adherence among individuals that are overweight/obese.

Stretch-shortening cycle potentiation (SSCP) is one such factor that enhances locomotion economy, via increased utilization of elastic energy that leads to greater force-generation (Roberts, 2002). Prior research investigating SSCP has shown the muscle-tendon complex of the ankle joint is stretched during single support; then rapidly recoils during the push-off phase (Kubo et al., 1999, 2000; Fukunaga et al., 2001, 2002). Thus, the muscle-tendon complex of the ankle joint acts as a spring-like mechanism through the storage and release of elastic-strain energy during locomotion. It is known (Kurokawa et al., 2001) that longer achilles tendons have higher compliance in the muscle-tendon complex and greater ability to store and utilize mechanical energy. Thus, the spring-like effect of the muscle-tendon complex may reduce the energy needed for muscle shortening during locomotion. We have also reported that Achilles tendon length is positively associated with improved walking economy (McCarthy et al., 2006) - potentially through enhanced SSCP. Consistent with this premise, we have previously shown reduced net oxygen uptake ($\dot{V}O_2$) during running is positively associated with SSCP at the knee (Hunter et al., 2015). However, little is known about the relationship between SSCP at the knee and walking economy. This is specifically important for older populations, where there is an increased reliance on proximal muscles versus distal muscles for mobility (Silder et al., 2008).

Increased muscle strength is related to the generation of larger eccentric force of a stretch shortening cycle (McCarthy et al., 2012). Previously, we have shown that locomotion velocity is positively associated with large forces exerted during the late eccentric phase of a stretch shortening cycle (McCarthy et al., 2012). It is therefore plausible that at least one reason why resistance training increases walking and running economy is due to enhanced SSCP. This possibly occurs in young adults since a youthful muscle-tendon complex may possess greater elasticity, and thus, more potential for generating force through SSCP. Although adults lose elasticity with advancing age (Hsiao et al., 2015; Niyomchan et al., 2019), there is evidence that older adults may use SSCP to the same degree as young adults for the plantar flexor muscles (Svantesson and Grimby, 1995). This may, in part, explain the findings of improved walking economy following resistance training in older adults (Parker et al., 1996; Hartman et al., 2007; Fisher et al., 2013). However, the effect of resistance training on SSCP in older adults is currently unknown.

Therefore, the purpose of this study was to determine whether combined aerobic and resistance exercise training increases SSCP in postmenopausal women over the age of 60 years. Since little is known concerning the relationship between SSCP and walking economy, a secondary objective was to examine this relationship. We hypothesized that SSCP will increase following 16 weeks of combined aerobic and resistance training, whereas SSCP will be positively related to walking economy.

MATERIALS AND METHODS

Participants

The present work is a secondary analysis of a study designed to delineate the effects of combined aerobic and resistance training following three different exercise frequencies over 16 weeks (Hunter et al., 2013). Initially, 92 postmenopausal women between 60 and 74 years of age provided baseline measures, wherein 76 participants completed the program. All participants were non-smokers and exercised less than one time per week, which was self-reported obtained during screening. Written informed consent was acquired from each participant prior to study involvement. All procedures confirmed to the guidelines set forth by the local (University of Alabama at Birmingham) institutional review board. Participants were randomly assigned to one of three training protocols: (a) Group 1: 1 d · wk^{-1} of resistance training and 1 d · wk^{-1} of aerobic training (1 + 1, n = 23); (b) Group 2: 2 d · wk^{-1} of resistance training and 2 d · wk^{-1} of aerobic training (2 + 2,n = 30) or; (c) Group 3: 3 d · wk^{-1} of resistance training and 3 d · wk^{-1} of aerobic training (3 + 3, n = 23).

Body Composition

In accordance with customary procedures and manufacturer specifications, total body fat percent was estimated before and after the training program using dual-energy x-ray absorptiometry (Lunar DPX-L densitometer; LUNAR Radiation, Madison, WI, United States). Adult Software v1.33 was used to analyze scans.

Resting Oxygen Uptake

Following an overnight fast between 1530 pm and 0800 am, resting $\dot{V}O_2$ was measured via open circuit, indirect calorimetry

system (DeltaTrac II: Sensor Medics, Yorba, CA, United States). The concluding 20 min of data were used for subsequent analyses.

Maximal Aerobic Capacity and Walking Economy

A modified Balke treadmill test coupled with a metabolic cart (Max-1 Cart; Physio-Dyne Instrument Corporation, Quogue, NY, United States) was used to measure maximal aerobic capacity ($\dot{V}O_{2max}$). A 12-lead electrocardiogram and blood pressure measures (Omron Blood Pressure Monitor, model HEM-780; Omron Healthcare, Inc., Bannockburn, IL, United States) were taken at 2 min intervals during the test and recovery period. Under physician supervision, the treadmill test began with 2 min of walking at 0.89 m/s (2 mph). Grade was increased by +3.5% every 2 min until min 12 wherein grade was decreased to 12% and speed was increased to 1.34 m/s (3 mph). Later grade was increased again by +2.5% each min until exhaustion. Maximal heart rate, respiratory exchange ratio (RER), and $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) were recorded as the highest 20 s averaged value.

On a separate day, walking economy was measured during a submaximal treadmill test (i.e., walking at 0.89 m/s). A walking speed of 0.89 m/s allowed us to achieve steady state within 4 min and compare walking economy at identical workloads. The 60 s mean value from the 3rd and 4th min were averaged to determine $\dot{V}O_2$. If $\dot{V}O_2$ and/or heart rate increased during the 4 min – a 5th min was used. Net $\dot{V}O_2$ was calculated by subtracting resting $\dot{V}O_2$ from steady-state $\dot{V}O_2$.

Maximum Muscle Strength Assessment

Participants performed a one-repetition maximum (1RM) test after the initial two exercise sessions (to permit overall familiarization). Notably we have previously revealed a high test-retest reliability for measurements conducted in our lab involving strength assessments (Hunter and Treuth, 1995). Determination of strength included the leg press, squats, leg extension, leg curl, elbow flexion, lateral pull-down, bench press, and military press. Lower back extension and bent leg sit-ups were performed with no weight according to section "Materials and Methods" previously described (Hunter and Treuth, 1995).

Stretch-Shortening Cycle Potentiation

A ballistic leg press, corresponding with 100% total body weight used as resistance, respective for each participant (McCarthy et al., 2012), was performed pre- and post-training. Concentric (CO) velocity during a *static* leg press throw was performed by instructing participants to slowly lower the sled to 90° knee flexion and hold the position for 3 s before pushing (i.e., extending the knees) the sled as fast as possible for maximal force. Concentric velocity was also measured during a counter-movement (CM) leg press throw by instructing participants to lower the sled to 90° knee flexion then immediately push sled off feet as fast as possible. The leg press sled was connected to a linear position transducer which was synced with a National Instruments system with a customized LabVIEW (Laboratory Virtual Instrument Engineering Workbench, version 7.1) software connected to a 16 channel 12-bit data acquisition system. Before each testing, calibration of linear position transducer was performed. Data was collected at 1 kHz. A low-pass fourth order Butterworth filter was applied with a cutoff frequency of 50 Hz. The linear position transducer tracks the linear position of the leg press sled. Using finite-difference technique the displacement data of sled was used to calculate velocity (McCarthy et al., 2012). The difference between CO and CM was representative of SSCP.

Supervised Exercise Training

Participants completed a 3–4 min warm-up on a treadmill or cycle ergometer followed by 3–4 min of stretching prior to each exercise session. All training sessions were supervised by an exercise physiologist in a facility dedicated to research. Each session lasted ≈50 min. The mode of aerobic exercise included a treadmill or cycle ergometer with at least 50% of training completed on the treadmill. Week one commenced with 20 min of continuous aerobic exercise corresponding with ≈67% of maximal heart rate (MHR). Weekly volume of aerobic exercise volume was progressively increased by modifying the duration first followed by intensity. For instance, duration was increased in weeks 2–5 by 5 min each week to reach a total of 40 min per session. At week 6, intensity was increased to ≈71% MHR, while duration decreased to 30 min. Duration then returned to 40 min by week 7. In week 8, intensity increased to ≈75% MHR while duration decreased to 30 min. At week 9, duration returned to 40 min. Intensity increased to ≈80% of MHR for 30 min by week 10. In week 11, duration increased to 40 min. For the remaining sessions, participants trained at ≈80% of MHR for 40 min. For subjects who did an aerobic and resistance workout same day, order of aerobic and resistance was alternated on their subsequent session. Rest interval between the sessions of aerobic and resistance training was 5–10 min and for resistance training it was 1–2 min between each set.

The resistance training protocol began with two sets of 10 repetitions at an intensity matching 60% 1RM with 90 to 120 s rest period between sets. Exercises included leg press, squats, leg extension, leg curl, elbow flexion, lateral pull-down, bench press, military press, lower back extension, and bent leg sit-ups. Resistance training progressed to 80% 1RM at week 8 (Hunter and Treuth, 1995).

Statistical Analyses

Baseline descriptive measures are reported as average ± SD. Effects on CO velocity, CM velocity, $\dot{V}O_2$ max and net $\dot{V}O_2$ from the three exercising training frequencies incorporating combined aerobic and resistance training were determined via two-way (group by time) repeated-measures analysis of variance (ANOVA). A One-way ANOVAs was used to compare age, height, body mass, body fat%, and heart rate. Multiple linear regression analysis (enter method) was used to determine associations of walking economy with groups, SSCP, and relative exercise intensity (%$\dot{V}O_{2peak}$) during the walking task at baseline and post-training. Because of equipment malfunction, there is

missing data for variables such as CM velocity, CO velocity, $\dot{V}O_{2max}$, and net $\dot{V}O_2$. Paired *t*-tests were run to analyze differences in SSCP for each respective groups. Degrees of freedom were calculated to reflect missing data. The threshold of statistical significance was set *a priori* with the *p*-value ≤ 0.05 for all analyses.

RESULTS

Baseline descriptive measures are shown in **Table 1**. Pre-/post-training data, including mean and SD's, for all study variables are presented in **Table 2**. There were no differences in age [$F(2,73) = 1.8$] or height [$F(2,73) = 1.2$] across the three exercise training groups. Body mass was significantly decreased [Time $F(1,71) = 4.24$, Group $F(2,71) = 4.51$, Time × Group $F(2,71) = 3.49$] post-training. Body fat percent was significantly decreased [Time $F(1,71) = 32.0$, Group $F(2,71) = 4.0$, Time × Group $F(2,71) = 2.7$] post-training, however no time by group interactions were observed. Maximal aerobic capacity, as evidenced by $\dot{V}O_{2max}$ was significantly increased [Time $F(1,65) = 8.6$, Group $F(2,65) = 3.7$, Time × Group $F(2,65) = 1.4$) among all groups. Likewise, walking economy (i.e., net $\dot{V}O_2$) [Time $F(1,69) = 12.5$, Group $F(2,69) = 0.12$, Time × Group $F(2,69) = 0.95$] and heart rate [Time $F(1,69) = 63.6$, Group $F(2,69) = 1.5$, Time × Group $F(2,69) = 0.95$] decreased similarly between groups across time. Despite no apparent time by group interactions for CO velocity [Time $F(1,65) = 6.5$, Group $F(2,65) = 0.08$, Time × group $F(2,65) = 2.87$] and CM velocity [Time $F(1,65) = 19.0$, Group $F(2,65) = 0.3$, Time × Group $F(2,65) = 0.2$, $df = 65$], a significant time effect ($p < 0.01$ for both) was revealed for all the groups. Paired *t*-tests analyses indicated a significant increase in SSCP in Group 2 only (**Figure 1**; Group 2, $p = 0.03$; Groups 1 and 3, $p > 0.5$).

Multiple linear regression of baseline measures ($n = 92$; **Table 3**) showed SSCP and relative exercise intensity (%$\dot{V}O_{2max}$) were independently related to net $\dot{V}O_2$ while walking at 2 mph ($r_{partial} = -0.22$, $p < 0.04$; and $r_{partial} = 0.54$, $p < 0.01$; respectively). However, only %$\dot{V}O_{2max}$ was significantly related to net $\dot{V}O_2$ post-training after adjusting for different exercise training groups ($r_{partial} = 0.49$, $p < 0.01$). No relationship was observed between SSCP and %$\dot{V}O_{2max}$ post-training ($r_{partial} = 0.18$, $p = 0.12$).

TABLE 1 | Participant characteristics at baseline ($n = 92$).

Characteristic	Mean ± SD
Age (year)	65 ± 4
Height (m)	1.65 ± 0.06
Body mass (kg)	73.8 ± 11.4
Body fat (%)	42.6 ± 6.1
$\dot{V}O_{2max}$ (mL · kg^{-1} · min^{-1})	22.8 ± 4.6
CM velocity (ms^{-1})	0.90 ± 0.12
CO velocity (ms^{-1})	0.77 ± 0.12
net $\dot{V}O_2$ (mL · kg^{-1} · min^{-1})	7.3 ± 1.28

CM, countermovement; CO, concentric only; $\dot{V}O_2$, oxygen uptake.

DISCUSSION

The primary objective was to determine whether combined aerobic and resistance exercise training increases SSCP in postmenopausal women over the age of 60 years. A secondary point of interest was to determine whether SSCP was related to walking economy (i.e., net $\dot{V}O_2$) at 0.89 m/s. Results revealed, independent of weekly volume, that 16 weeks of combined exercise training significantly improved both walking economy and ease (i.e., ↓heart rate). Although participants exercising 1 d · wk^{-1} and 3 d · wk^{-1} did not increase SSCP, significant improvement was detected among participants exercising 2 d · wk^{-1}. Of note, SSCP was related to walking economy at baseline suggesting that the ability to use elastic energy, as evidenced by SSCP, may partially lower the energetic requirements for non-graded walking. These findings are consistent with previous work in our lab wherein SSCP related to non-graded walking (unpublished data) and running economy in young (≈31 year) men (Hunter et al., 2015). To our knowledge, we are the first to show the relationship of SSCP to walking economy in an older population. However, we were surprised to discover this relationship did not persist following exercise training, thus we offer several possible explanations for this observation.

Whereas post-training walking economy was increased among all groups, no relationship between SSCP and walking economy was noted – yet this is not entirely unexpected. A possible explanation involves the muscle groups/patterns used during non-graded walking in older individuals. Stretch-shortening cycle potentiation was measured incorporating a leg press throw, a task primarily executed from the hip and knee extensors. Hence, in this study, it is probable that the technique used to measure SSCP was primarily developed in the hip/knee extensors (as opposed to the plantar flexors). Evidence suggests older adults tend to be more dependent on hip extension and less on plantar flexion during walking tasks compared to younger adults (Silder et al., 2008), a feature likely due to reduced strength in the plantar flexor muscles (Silder et al., 2008; Anderson and Madigan, 2014). Given that hip/knee extensors bioenergetics are less efficient than plantar flexors, greater dependence on hip/knee extension may contribute to an increase in energy expenditure (Seki et al., 2019). Though speculative, the improved muscle strength post-training may have enabled the older adults to adopt a walking pattern that more closely resembles that of younger adults (i.e., shift in the involvement of distal muscles and respective reliance on elastic energy from distal muscle-tendon units).

There is evidence that indicates older adults have insufficient range of motion at the hips which could contribute to the known age-related deficits in gait (Anderson and Madigan, 2014). It is possible that the combined aerobic and resistance training could have positively affected hip/knee range of motion (ROM), thus enhancing walking economy, but this is only speculative as no direct measures were taken of ROM. Based on these factors, it is reasonable that the potential link between SSCP (reflective of knee/hip extensors) and walking economy would be expected to diminish post-training. Moreover, effects of exercise training are

TABLE 2 | Differences in physiological and functional parameters for each respective groups.

Parameters	Group 1[a] *n* = 23			Group 2[b] *n* = 30			Group 3[c] *n* = 23			
	Pre	**Post**	***% diff***	**Pre**	**Post**	***% diff***	**Pre**	**Post**	***% diff***	***p*-value**
Age (yr)	66 ± 4			64 ± 3			64 ± 3 (*n* = 21)			*G* = 0.17
Height (cm)	167 ± 6			165 ± 5			164 ± 4 (*n* = 21)			*G* = 0.31
Body mass (kg)	76.3 ± 11.4	76.4 ± 11.4	0.13	74.9 ± 9.4	73.7 ± 9.1	1.6	67.8 ± 9.6 (*n* = 21)	67.60 ± 9.7	0.29	*T* < 0.05* *G* < 0.02* G×T < 0.04
Body fat (%)	44.1 ± 5.3	43.6 ± 5.7	1.13	42.8 ± 4.7	41.1 ± 4.8	3.97	40.1 ± 5.8 (*n* = 21)	38.5 ± 6.7	3.99	*T* < 0.01* *G* = 0.02* G×T = 0.07
$\dot{V}O_{2max}$(mL · kg^{-1} · min^{-1})	21.0 ± 2.9	21.7 ± 3.7	3.33	23.0 ± 4.6 (*n* = 26)	24.9 ± 3.8	8.2	24.0 ± 4.3 (*n* = 19)	24.5 ± 5.1	2.08	*T* < 0.01* *G* = 0.03 G×T = 0.25
CM velocity (m.s^{-1})	0.89 ± 0.12 (*n* = 22)	0.93 ± 0.12	4.49	0.91 ± 0.10 (*n* = 25)	0.96 ± 0.10	5.5	0.88 ± 0.11 (*n* = 21)	0.93 ± 0.13	5.68	*T* < 0.01* *G* = 0.73 G×T = 0.82
CO velocity(m.s^{-1})	0.78 ± 0.13 (*n* = 22)	0.80 ± 0.12	2.56	0.78 ± 0.10 (*n* = 25)	0.78 ± 0.13	0.0	0.75 ± 0.14 (*n* = 21)	0.81 ± 0.11	8.0	*T* < 0.01* *G* = 0.61 G×T = 0.21
net $\dot{V}O_2$(mL · kg^{-1} · min^{-1})	7.5 ± 1.5	6.6 ± 0.9	12.0	7.3 ± 1.3 (*n* = 29)	6.7 ± 1.5	8.22	7.1 ± 1.1 (*n* = 20)	6.7 ± 1.5	5.63	*T* < 0.01* *G* = 0.89 G×T = 0.64
Heart rate(beats · min^{-1})	108 ± 14	97 ± 8	10.18	101 ± 14 (*n* = 26)	94 ± 14	6.93	101 ± 12	94 ± 10	6.93	*T* < 0.01* *G* = 0.23 G×T = 0.39

*CM, countermovement; CO, concentric only; $\dot{V}O_2$, oxygen uptake. Data are presented as means ± standard deviations. *p < 0.05. Group differences (G); Time differences (T); Group × Time Interaction (G × T). [a]1 d · wk^{-1} of resistance training and 1 d · wk^{-1} of aerobic training; [b]2 d · wk^{-1} of resistance training and 2 d · wk^{-1} of aerobic training, and [c]3 d · wk^{-1} of resistance training and 3 d · wk^{-1} of aerobic training. %diff, percent difference.*

TABLE 3 | Multiple linear regression model of walking net $\dot{V}O_2$ versus stretch-shortening cycle potentiation (SSCP) and relative exercise intensity (%$\dot{V}O_{2max}$) at baseline (*n* = 92) and post-training (*n* = 68).

Independent variable	Dependent variable	R^2	Intercept	Slope	$r_{partial}$	*p-value*
Model 1: Baseline net $\dot{V}O_2$ walk (mL · kg^{-1} · min^{-1})		0.32	5.0			<0.01
	SSCP			−2.88	−0.22	<0.04
	Percentage $\dot{V}O_{2max}$			6.00	0.54	<0.01
Model 2: Post-training net $\dot{V}O_2$ walk (mL · kg^{-1} · min^{-1})		0.25	3.2			<0.01
	SSCP			2.37	0.18	0.12
	Percentage $\dot{V}O_{2max}$			7.84	0.49	<0.01

$\dot{V}O_2$, oxygen uptake; SSCP, stretch-shortening cycle potentiation. No relationship with groups were found.

dictated by multiple factors which can affect within-individual response to different types of exercise training (Hecksteden et al., 2015). Further examination of factors including altered biomechanics and change in achilles tendon compliance (Kooper et al., 2014) in various study designs such as crossover design should be considered to further refine our understanding linking SSCP and walking economy.

Indeed, both ease of walking and economy were improved following 16 weeks of combined aerobic and resistance training in the present study. Since all the participants performed a combination of both aerobic *and* resistance training, we are unable to differentiate which mode of exercise training is better suited to enhance ease of walking and economy. This is in line with prior evidence showing positive effects on walking economy due to multicomponent exercise training (Valenti et al., 2016). Both these modes of exercise, aerobic and resistance training, can lead to improved mitochondrial profile and function (Nilsson and Tarnopolsky, 2019), which may improve walking economy. However, there is proof that positive relationship exists between stage 3 respiration (coupled respiration) and stage 4 respiration (uncoupled respiration) with both, pre and post aerobic training, suggesting that mitochondrial function has little effect on walking

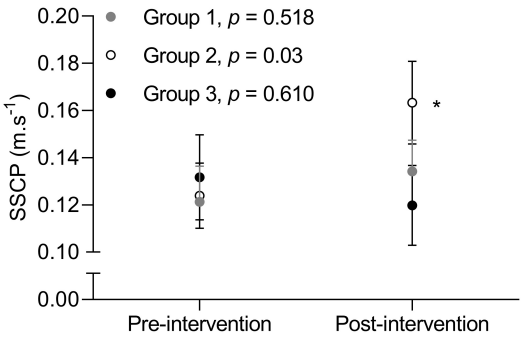

FIGURE 1 | Mean ± standard error of group values for stretch-shortening cycle potentiation (SSCP) captured by the pre-training versus post-training difference in a concentric only and counter-movement leg press throw. Note the significant increase in group 2 where: Group 1: 1 d · wk^{-1} of resistance training and 1 d · wk^{-1} of aerobic training (n = 23); Group 2: 2 d · wk^{-1} of resistance training and 2 d · wk^{-1} of aerobic training (n = 30); Group 3: 3 d · wk^{-1} of resistance training and 3 d · wk^{-1} of aerobic training (n = 23). *p = 0.03.

economy due to aerobic training (Hunter et al., 2005, 2019). These findings may suggest that factors other than mitochondrial efficiency factors influence improvements in walking economy following exercise training.

Co-contraction of antagonist muscles is inversely related with walking economy in older adults (Ortega and Farley, 2015). Exercise training can reduce antagonist muscle activation in older adults (Hakkinen et al., 1998), however it is undecided if reduced co-contraction of antagonist muscles can improve walking economy in older adults (Beck et al., 2018). We did not assess co-contraction of muscle in our current study. Notably, several studies have shown increased ease of walking and economy with resistance training – independent of improved aerobic capacity (Parker et al., 1996; Hartman et al., 2007). Further evidence involving changes in muscle strength following resistance training have been associated with increased walking economy (Hunter et al., 2008). Thus, our findings underline the importance of combined aerobic and exercise training to elicit meaningful improvements in ease of walking and economy - particularly among older adults. This information has important clinical application/utility as ease of walking and economy are associated with both increased free-living energy expenditure (Hunter et al., 2000, 2013) and longer-term accretion of body fat (Weinsier et al., 2002). Certainly these data offer a framework for further investigation to incorporate exercise training as a means to augment ease of walking and economy. This, in turn, may enhance spontaneous engagement in PA among older adults.

We were not entirely surprised with our finding of increased SSCP only with group 2. A recent meta-analysis suggested that two exercise training sessions consisting of resistive exercises per week is most efficient for improving muscle strength in older adults (Borde et al., 2015). Since muscle strength can, in part, dictate improvements in SSCP, it may explain increased SSCP in Group 2 only. Further, effects of exercise could be sex-dependent (Hunter and Treuth, 1995). Older women (who were the study population of our study) may respond better to moderate exercise frequency (Grgic et al., 2018). Notably, we used a combination of aerobic and resistance training. There is lack of data on effects of different types of exercise training, specifically a combination of aerobic and resistance training, on SSCP. Future studies should examine effects of different types of training with different frequency/volume on SSCP in older women.

CONCLUSION

Among older postmenopausal women, our results indicate that irrespective of frequency of training, 16 weeks of combined aerobic and resistance exercise training increased ease of walking and economy. Additionally, only participants exercising 2 d · wk^{-1} exhibited significant improvement in SSCP. Baseline measures of SSCP were associated with walking economy independent of relative exercise intensity, however, these results did not persist post-training. Individual responsiveness to exercise training and shifts in muscle involvement/patterns may have contributed to these findings.

DATA AVAILABILITY STATEMENT

The datasets related to this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board, University of Alabama at Birmingham. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GH, JM, and DB were responsible for study design and data acquisition. All authors helped with data analysis and data interpretation, critically revised the manuscript, contributed with important intellectual content, approved the final version of the article to be submitted for publication purpose, and took responsibility for appropriate portions of the content. HS, GH, and SM wrote the first draft of the manuscript.

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REVIEW
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Stretching Interventions in Children With Cerebral Palsy: Why Are They Ineffective in Improving Muscle Function and How Can We Better Their Outcome?

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Hyper-resistance at the joint is one of the most common symptoms in children with cerebral palsy (CP). Alterations to the structure and mechanical properties of the musculoskeletal system, such as a decreased muscle length and an increased joint stiffness are typically managed conservatively, by means of physiotherapy involving stretching exercises. However, the effectiveness of stretching-based interventions for improving function is poor. This may be due to the behavior of a spastic muscle during stretch, which is poorly understood. The main aim of this paper is to provide a mechanistic explanation as to why the effectiveness of stretching is limited in children with CP and consider clinically relevant means by which this shortcoming can be tackled. To do this, we review the current literature regarding muscle and tendon plasticity in response to stretching in children with CP. First, we discuss how muscle and tendon interact based on their morphology and mechanical properties to provide a certain range of motion at the joint. We then consider the effect of traditional stretching exercises on these muscle and tendon properties. Finally, we examine possible strategies to increase the effectiveness of stretching therapies and we highlight areas of further research that have the potential to improve the outcome of non-invasive interventions in children with cerebral palsy.

Keywords: muscle, tendon, *in vivo*, sarcomere addition, stiffness

INTRODUCTION

Cerebral palsy (CP) is a non-progressive disorder caused by a brain lesion taking place in the early stages of development (Graham et al., 2016). The neurological lesion in CP causes adaptations in the muscle, including muscle atrophy (Shortland, 2009), fibrosis (Booth et al., 2001), muscle shortening (Barrett and Lichtwark, 2010) and overstretched sarcomeres (Mathewson et al., 2014). The combination of longer sarcomeres with shorter muscle fibers mean that there will be fewer in-series sarcomeres (Mathewson et al., 2014). Additionally, there is a lack of muscle growth (Gough and Shortland, 2012; Willerslev-Olsen et al., 2018). This dynamic shortening of the muscles

is typically treated with stretching exercises, botulinum toxin injections, casting or ankle-foot-orthoses. Eventually, if these treatments are not sufficiently effective fixed contractures can develop, which is treated with surgery.

One of the main aims of these treatments which try to maintain or increase ROM is to improve the gait pattern. An increase in muscle-tendon unit (MTU) length could be gained by permanently stretching existing structures, however, this is probably less desired as a carry-over effect to function may not occur. To improve functionality, the increase in length should be rather achieved by building new contractile material in-series within the MTU. Ideally, we want to promote large, strong and flexible muscles that allow contractile function across the muscle's full range of motion. Therefore, interventions should aim to increase fibers length by promoting serial sarcomerogenesis. According to the force-length characteristics of muscle (Lieber, 2002), such an increase in muscle fibers length would allow for force production over the newly acquired ROM.

For the purpose of increasing ROM, different stretching methods have been used. Passive or active stretches can be applied either manually by a clinician or by the patients themselves as initial conservative treatments. Ankle-foot-orthoses or casting are used to hold the joint near their end-range of motion to provide a more chronic stretch of the muscle. By increasing ROM, stretching methods should consequently better daily function, delay the development of contractures and the need for surgical intervention This relies on the assumption that the muscle is able to generate forces throughout a larger excursion used over the increased ROM. The stretching exercises can cause discomfort to the children, they are time consuming for the patient their families (Hadden and Von Baeyer, 2002) and the physiotherapists (Wiart et al., 2008). Additionally, there is limited evidence to support functional improvements, e.g., in gait and mobility, after stretching interventions (Preissner, 2002; Katalinic et al., 2011; Harvey et al., 2017). Clearly a significant gap exists between the clinical rationale for implementing stretching and the supporting evidence for its intended effectiveness. Despite this, stretching remains a widely prescribed method of treatment, probably due to the comparative simplicity and safety of its application as a first line treatment. Therefore, the main aim of this paper is to review the evidence and provide a mechanistic explanation as to why the effectiveness of stretching is limited in children with CP and consider clinically relevant means by which this shortcoming can be tackled.

To do this, we will first discuss how the muscle and its tendon interact in children with CP based on their morphology and mechanical properties to limit ROM at the joint. This discussion will be presented in terms of a simplified muscle model of the medial gastrocnemius. The discussion will focus on the contribution of different structures within the MTU to the ROM and consider whether the fibers experience an adequate stretching stimulus to promote adaptation. Second, we will consider the effect of traditional stretching exercises on the muscle and tendon properties in children with CP by examining the literature about both manual passive stretching, ankle-foot-orthoses and lower-leg casts on muscle and tendon properties. Finally, we will examine possible solutions to increase the effectiveness of stretching therapies and highlight areas of further research that have the potential to improve the outcome of non-invasive interventions for children with CP.

MUSCLE AND TENDON PROPERTIES IN CP

The mechanical properties of a muscle dictate the degree of its length alteration in response to an applied force. This response depends partly on the geometry of the muscle, as well as on the intrinsic tissue material properties. In this paper we will discuss the changes that occur to the architecture and the mechanical properties of the medial gastrocnemius muscle in children with CP. To do this, it is common to use a simplified pennate muscle model (**Figure 1**) of the medial gastrocnemius. In this model, the MTU consists of a muscle and a tendon placed in series, where the changes in the muscle belly length are dependent on changes of the muscle fascicles and the pennation angle. The medial gastrocnemius muscle is most commonly studied in children with CP for a few reasons: it is a superficial muscle, therefore suitable for most imaging techniques and this muscle is essential for functional tasks such as walking. The architectural structure of muscles can be studied in a straightforward manner with medical imaging techniques such as MRI (e.g., Elder and Kirk, 2003), and 2D (e.g., Shortland et al., 2002) or 3D ultrasound (e.g., Cenni et al., 2018a; Cenni et al., 2018b, while the mechanical properties (stiffness) of a muscle can be assessed by applying a known tensile force to the tissue and measuring the resultant elongation (Maganaris, 2002). Due to anatomical constraints, some assumptions need to be made when quantifying *in vivo* muscle and tendon stiffness. For example, when applying a passive moment around the ankle joint to dorsiflex the foot, the lengthening of the medial gastrocnemius MTU achieved depends on the MTU's moment arm length (Kalkman et al., 2017) the properties of all agonist plantarflexor and antagonist dorsiflexor MTUs acting around the ankle joint, and the properties of all passive structures within the joint (e.g., ligaments and joint capsule) These factors might vary with ankle angle, making it difficult to quantify the passive force carried by MTU during passive joint rotation. Alternatively, instead of calculating muscle stiffness in absolute terms, we can estimate the relative contribution of muscle and tendon to the joint's ROM as the muscle and its tendon are placed in series and therefore the passive force along these two structures is expected to be the same.

Muscle Architecture in CP

There is consistent evidence that the medial gastrocnemius muscle is shorter in the paretic leg of children with CP compared to matched typically developing children (Fry et al., 2007; Malaiya et al., 2007; Barrett and Lichtwark, 2010). This is accompanied by a greater length of the Achilles tendon in children with CP (Wren et al., 2010; Barber et al., 2012). Furthermore, some studies have reported reduced muscle fascicle lengths at rest in children with CP than in TD children (Mohagheghi et al., 2008; Matthiasdottir et al., 2014; Kalkman et al., 2018b;

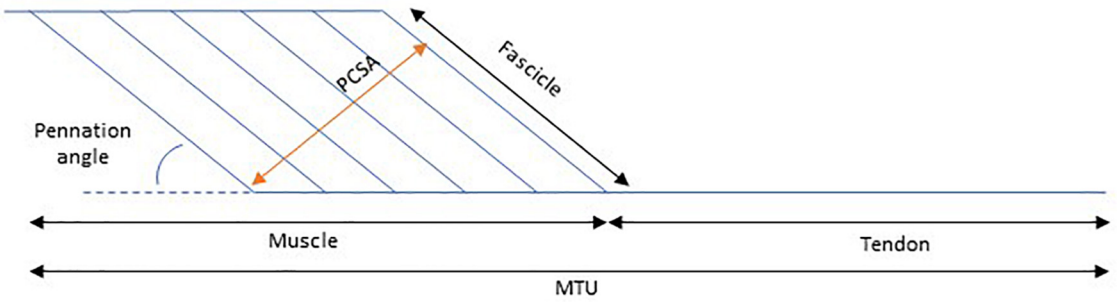

FIGURE 1 | Schematic representation of the contribution of muscle length to muscle tendon unit (MTU) length. PCSA, Physiological cross-sectional area.

D'Souza et al., 2019; Frisk et al., 2019), but others have not detected differences (Shortland et al., 2002; Malaiya et al., 2007).

These findings can have significant functional consequences. Shorter muscle fascicles with fewer sarcomeres in series, as found in children with CP (Lieber and Fridén, 2002; Ponten et al., 2007; Mathewson et al., 2014), will have a limited potential for active shortening. This, would in turn influence the force generating capacity of the muscle (Frisk et al., 2019). It has also been shown that the force-length relation of muscles in children with CP was different to that of typically developing children, with maximal torque generation occurring at a more plantar flexed position (Frisk et al., 2019). This may be a contributing factor to gait impairments such as toe-walking, the more plantarflexed joint position during gait would take advantage of the altered moment-angle relationship. These findings highlight that it is essential to increase fibers length and extensibility to improve the force-length relation of the muscle, firstly with respect to the position at which maximal torque is produced and secondly with respect to the range over which force can be produced (Lieber and Friden, 2000).

Lengthening Behavior of the Muscle

For an increase in fibers length to occur in response to a stretching intervention, it is essential that the muscle fibers receive an adequate stretching stimulus. The amount of stretch that the muscle experiences is dependent on the mechanical properties of both the contractile tissue as well as the connective tissue within the muscle fibers and the tendon. It has been shown that, when the ankle joint is passively dorsiflexed *n* children with CP, the belly of medial gastrocnemius muscle elongates less compared to TD children (Matthiasdottir et al., 2014). Additionally, some studies have reported lengthening of the muscle fascicles to be unaltered during passive joint rotation (Matthiasdottir et al., 2014), while others show a reduction in fascicle lengthening in CP when compared to TD children (Barber et al., 2011; Kalkman et al., 2018b). These inconsistent findings could potentially be accounted for by different ways of comparing groups. A reduced ROM in children with CP could complicate comparisons made over the full ROM. Additionally, due to differences in Achilles tendon moment arm length (Kalkman et al., 2017) and joint stiffness (Alhusaini et al., 2010) between TD and CP participants, comparisons in terms of joint angles should be interpreted with caution. To circumvent this problem, fascicle lengthening during passive joint rotations has been additionally studied over a common torque range and a common MTU lengthening, where it was found that irrespective of the method used the stiffness of the muscle fascicles relative to their in-series tendon was higher in children with CP (Kalkman et al., 2018b).

Due to the above-mentioned changes in muscle and tendon properties, the stretch "seen" by the muscle and the fascicles would be reduced in children with CP. It has been shown in rabbits that strain experienced by the muscle fibers is a more potent stimulus for in series sarcomerogenesis than MTU strain (Butterfield and Herzog, 2006). Therefore, it is reasonable to assume that the reported alterations to muscle and tendon properties play a crucial role for the response to stretching interventions, as is expected in TD individuals (Morse et al., 2008).

THE EFFECT OF STRETCHING ON MUSCLE AND TENDON PROPERTIES IN CHILDREN WITH CP

Muscle stretching, in the form of passive stretching exercises, orthotics, casting, standing tables, or a combination of these modalities, has been recommended in the early management of joint hyper-resistance in children with CP (Pin et al., 2006; Blackmore et al., 2007; Bovend'Eerdt et al., 2008; Novak et al., 2013). Here we assess the effect of traditional stretching exercises on muscle and tendon properties. Although there is an abundance of literature showing a positive effect of stretching exercises on ROM [as reviewed by Eldridge and Lavin (2016)], functional indexes such as gait parameters, walking velocity and gross motor function rarely improve (Craig et al., 2016). This may be because the muscle and the tendon do not adapt in the required ways, a concept that has not been studied systematically.

During typical growth and development there is an age-dependent adaptation of muscle and tendon in response to stretch. It has been suggested that during normal growth, both the muscle and the tendon grow, and the tendon grows at a slightly lower rate (Wren, 2003). Younger animals have been shown to adapt to immobilization by increasing tendon length, where older animals add sarcomeres in-series (Tardieu et al., 1977). The use

of a computational model suggested that the tendon acts like this at young age in order to minimize tensile strains (Wren, 2003). Growth factors that are altered during development may be responsible for these different muscle and tendon adaptation across the lifespan (Okamoto et al., 2005). In children with CP this is additionally complicated by the fact that growth factor seems to be further altered from typical (Von Walden et al., 2018; Pingel et al., 2019). This raises the question whether it is the muscle or the tendon adapting in younger and older children with CP. Are the muscles able to increase length by sarcomerogenesis or is the tendon additionally lengthened by stretching treatments?

Passive Stretching

Passive stretching exercises are carried out by a therapist without the patient's own muscular involvement. The muscle to be stretched is lengthened as the therapist manually moves the joint of the patient at its end-range position. The therapist holds this end-range position for a certain amount of time. The effect of manual passive stretching on muscle and tendon properties has been investigated both acutely after a bout of stretching and after a long-term intervention. Acutely, passive stretching has been shown to increase ROM in children with CP (Theis et al., 2013; Kalkman et al., 2018a). Theis et al. (2013) performed passive stretches for five repetitions of 20 sec, either applied by a physiotherapist or by the children themselves. Kalkman et al. (2018a) performed three sets of five 20 sec repetitions of passive stretching, all applied by a physiotherapist. According to Theis et al. (2013) the increase in ROM was accounted for by an increase in length of all 3 structures that make up the MTU, muscle belly, fascicles and tendon. However, the average reported increase in MTU lengthening after stretching (18.5 mm) seemed extremely large for an increase in ankle ROM of only 9.8°. A later study showed that a similar increase in ROM (10°) achieved directly after a single bout of stretching resulted in only 3.9 mm average increase in MTU lengthening, where 80% of the increase in maximal MTU length was accounted for by the muscle fascicles and the remaining 20% could be attributed to the Achilles tendon and aponeurosis (Kalkman et al., 2018a). Kalkman et al. (2018a) additionally showed that the amount of length change in the muscle belly achieved by joint rotation over a common ROM was not altered. Therefore, they concluded that the increased ROM could be due to a greater torque applied passively by the examiner and thus an increased tolerance to stretch, dependent on several factors, including, pain tolerance, warm-up, and acquaintance between therapist and patient.

After a 6-week stretching intervention that consisted of 15 min stretching (10 repetitions of 60 sec) for four times a week, it was shown that ROM increased, joint stiffness decreased, resting fascicle length remained similar and muscle stiffness decreased (Theis et al., 2015). However, as mentioned above there are several limitations associated with measuring muscle stiffness, since the calculated force in the muscle depends on the MTUs moment arm, passive structures around the joint and other agonist/antagonist muscles. Nonetheless, the authors hypothesized that the changes in stiffness were due to changes in muscular connective tissue and not by an increase in serial sarcomere number (Theis et al., 2015). A similar 6-week stretching intervention was performed by Kalkman et al. (2019), who found an increase in ROM and no changes in resting fascicle length. Hence, similarly to the acute response (Kalkman et al., 2018a), it was hypothesized that the increase in ROM was caused by a chronic increase in stretch tolerance. This concept would also be able to explain the increase in fascicle strain as seen in Theis et al. (2015). Contrasting results were found by Hösl et al. (2018) who reported a small decrease in resting fascicle length after 9 weeks of passive stretching. However, fascicle strain did increase, which was attributed to larger passive dorsiflexion ROM and tolerated stretch-moments (Hösl et al., 2018). In comparison to Theis et al. (2013) and Kalkman et al. (2018a) the time period each stretch was held was shorter (20 s vs. 60 s), but the total intervention time was about 27% longer. The literature is still lacking studies looking at changes in the structure of individual muscle fibers (at sarcomere level) after stretching interventions, which could possibly clarify and explain the observations described above.

Ankle Foot Orthoses

An ankle foot orthosis (AFO) is a medical device that imposes a mechanical constraint to the ankle and the foot. The AFO can directly affect movement of the ankle both during gait and at rest (i.e., at night). AFO's are commonly used to hold the joint more permanently near the end range of motion applying constant, moderate stretch to the MTU. The only study found that reported the effect of AFO's on muscle/tendon structure in children with CP, showed little change in muscle belly and tendon length after 16 weeks of ankle-foot bracing (Hösl et al., 2015). However, a 11% decrease was found in fascicle length, which was suggested to be caused by a loss of sarcomeres in series (Hösl et al., 2015). Similar to the results described in the previous section, it seems that the muscle does not "see" much of the stretch stimulus in AFO's too, which might explain these findings of limited changes in muscle/tendon structure. The authors concluded that it seems difficult to change muscle morphometrics by single traditional treatments and there is a need for concomitant treatments to promote muscle growth (Hösl et al., 2015). Additionally, a simulation study showed that the operating length of the medial gastrocnemius MTU during gait is highly variable between individuals and types of AFO (Choi et al., 2016), suggesting that, with a proper orthotic design, it may be possible to promote in series sarcomerogenesis. Clearly, more focused research is required to identify the mechanisms of inter-subject variability in muscle remodeling in response to chronic stretching.

Serial Casting

Lower-leg casting refers to the application of plaster to immobilize the ankle joint. Serial or progressive lower-leg casting involves the successive application of a series of casts, placing the foot at gradually increasing dorsiflexion angles with each cast. In an initial study, which still requires full review, publication, and confirmation, the effects of 2 weeks of lower-legs casting only on the ankle joint as well as the underlying muscle and tendon properties, was examined in a group of children with CP (Peeters et al., 2018). It was shown that post-casting, an

increased maximum ankle dorsiflexion angle was accompanied by increased tendon length, rather than muscle length (Peeters et al., 2018). More studies are needed, especially to evaluate if the effects may differ depending on age, as shown in animals (Tardieu et al., 1977). If corroborated, these results will also be in line with the findings described above where the increase in joint ROM after the use of AFO's could be explained by an increase in tendon length (Hösl et al., 2015). Once again, this effect may be explained by the same principle applied during passive stretches, where the tendon "sees" most of the stretch (Kalkman et al., 2018b).

IMPROVING THE EFFECTIVENESS OF STRETCHING IN CP

As discussed above, stand-alone stretching methods applied manually, with AFO's or serial casting do not seem effectively improve the muscle and tendon. Adaptations seem to be either absent or in a negative direction after a period of stretching. In this context, a combination of treatments have been tried to increase the stretch stimulus to the muscle. In this section we will discuss the effect of different combination treatments on muscle and tendon properties.

Botulinum Toxin-A and Stretching

Common in clinical practice is the combination of Intramuscular Botulinum neurotoxin-A (BoNT-A) injections with stretching by serial casting. The hypothesis is that BoNT-A injections temporarily reduce the symptoms of excessive tonic discharge thus providing a "window of opportunity" during which adjunctive interventions such as serial casting and physiotherapy can be implemented to improve, or at least prevent further deterioration of, muscle structure and functionality (Franki et al., 2019). Therefore, when these are reduced, the muscle will experience more of the tensile stimulus applied by the adjunctive stretching intervention. The above reasoning goes hand in hand with the idea that contracture is due primarily to hyperactive stretch reflexes. However, the evidence for that is quite the contrary, with muscle alterations preceding the emergence of hyperactivity (Willerslev-Olsen et al., 2013, 2014). Nevertheless, it is assumed that when hyperactive stretch reflexes are reduced, the muscle will experience more of the tensile stimulus applied by an adjunctive stretching intervention. There is limited evidence that BoNT-A combined with rehabilitation is effective in altering muscle structure or preventing contracture (Boyaci et al., 2014), even though there are indications of short term improvement in joint ROM, gait and function, as measured by the goal attainment scale (Molenaers et al., 2013; Novak et al., 2013). Specifically, promising results have been observed in functional improvements, as measured by the Goal Attainment Scale, after combining BoNT-A with different kinds of stretching therapies (Molenaers et al., 2013). More research is needed to better understand how these functional improvement are achieved.

Evidence from animal studies suggest that BoNT-A may increase muscle stiffness, and that its long term use may cause detrimental levels of muscle atrophy (Gough et al., 2007; Pingel et al., 2016). Additionally, it was shown that muscle volume decreased significantly after BoNT-A injections (Williams et al., 2013b), usually an unwanted outcome that could be counterbalanced with strength training (Williams et al., 2013a). Together with a reduction in normalized muscle volumes, alterations to muscle quality were shown by a reduction in echogenicity in a cohort of children with who had received BoNT-A injections compared to a cohort of children with CP who did not receive any BoNT-A injections (Schless et al., 2019). Although no difference could be shown between single and multiple BoNT-A injections (Barber et al., 2013), it seems unlikely that multiple BoNT-A injections would promote muscle growth, the desired outcome from the stretching interventions discussed in this review.

Specific Collagenase Enzymes and Stretching

To avoid the negative effects of BoNT-A injections on the muscle, another way to reduce the stiffness of the spastic muscle has recently been proposed. It was hypothesized that injecting specific collagenase enzymes, to digest part of the extensive collagen in the extracellular matrix (Smith et al., 2011), which play a role in contractures would reduce muscle stiffness (Howard et al., 2019). This theory is about to be tested in a spastic mouse model, but it is hypothesized that if selective collagenase is injected into spastic muscle at an appropriate dilution and concentration in combination with a stretching program, it might lead to increased ROM and improvements in sarcomere length, hence enhancing force production over the newly acquired ROM (Howard et al., 2019).

Strengthening and Stretching

As described above, the aim of a combined treatment is to increase the stretching stimulus to the muscle. BoNT-A injections achieve this by a reduction in tonic discharge, that alters the relative lengthening of muscle and tendon during stretch (Bar-On et al., 2014). Alternative methods for the same purpose, have been proposed. For example, the relative lengthening of muscle to tendon during passive stretch could be increased when tendon stiffness is increased. It has been shown that tendon stiffness is adaptable to mechanical loading and can increase with resistance training in several populations, including adults (Couppé et al., 2008), elderly (Reeves et al., 2003) and typically developing pre-pubertal children (Waugh et al., 2014). A combined strengthening and stretching intervention was designed by Kalkman et al. (2019) to promote the stretch seen by the muscle. This intervention group was compared to a control group, who performed only stretching exercises. Heel raises were performed as progressive resistance training exercises to strengthen the ankle plantar flexor muscles. The target was to complete 3 sets of 12 repetitions four times a week, exercises were progressed by adding weight to a backpack. Muscle strength, as measured by a maximal voluntary contraction (MVC), increased significantly, as did Achilles tendon stiffness. As a result of this, the stretching stimulus to the muscle was increased. Consequently, resting fascicle length in the intervention group increased (average of 2.2 mm), while in the

control (stretching only) group it remained unaltered (average change of −0.5 mm). The authors thereby provided proof of concept that combining resistance with stretching training might be an effective intervention for increasing muscle fascicle length in children with CP. Additional analysis of fascicle lengthening during passive ankle rotation showed no pre to post changes in the control group. In the intervention group, however, there was a post-intervention shift toward greater dorsiflexion in the ankle angle at which the fascicles started to elongate when the ankle was moved passively (**Figure 2**). This is consistent with the fascicle length adaptation achieved and reinforces the notion that by increasing the stiffness of the tendon, the in-series fascicles can "see" more of the tensile stimulus applied during passive stretching and therefore adapt accordingly. However, the ROM improved similarly in the control and intervention groups, suggesting that stretch tolerance plays a more important role in limiting end range joint positions compared to in-series tissue mechanical properties. Functional benefits of an increased resting fascicle length on force production over the newly acquired ROM should be systematically and more directly investigated in the future.

Similar results were reported by Zhao et al. (2011) after passive-stretching and active-movement training. The training consisted of 20 min passive stretching, 30 min active movement and 10 min passive stretching. During the active movement training, a rehabilitation robot was used to actively engage the patients in computer games to exercise the ankle joint in both dorsi and plantar flexion directions. The passive stretching was performed by an ankle rehabilitation robot, which applied a predetermined torque to stretch the calf muscle toward end range dorsiflexion. After a 6-week training program, increases in Achilles tendon stiffness and medial gastrocnemius and soleus fascicle lengths across the ROM were reported. This study, however, does not report any comparisons to a control group, therefore it is difficult to draw conclusions about the mechanisms behind these observed changes.

Electrical Stimulation and Stretching

A different approach to improve the efficacy of stretching interventions was suggested by Khalili and Hajihassanie (2008). It was proposed that electrical stimulation of the antagonist muscle may improve the efficacy of stretching by providing an additional tensile stimulus to the agonist muscle and by reciprocally inhibiting the stretched muscle. The intervention consisted of 30 min electrical stimulation of the quadriceps muscle three times a week and passive stretching of the hamstrings muscle five times a week. The intervention was applied to one leg, while the opposite leg served as a control receiving only passive stretching to the hamstrings. Maximal passive knee extension was shown to increase more in the intervention leg compared to the control leg. The mechanisms through which electrical stimulation might lead to the above improvements were not studied. Moreover, the technical complexity and discomfort associated with this treatment approach might prevent it from becoming routine clinical practice for ambulant children with CP.

Eccentric Fascicle Loading

High mechanical stress and stretching to the muscle can also be achieved during eccentric strength training exercises. It has been shown that in healthy individuals eccentric plantarflexor training can promote strength and increase muscle fascicle length (Duclay et al., 2009). Backward-downhill treadmill training (BDTT) was suggested to provide such an eccentric fascicle loading on the gastrocnemius in children with CP (Hösl et al., 2016). Recently BDTT was compared to conventional stretching (Hösl et al., 2018). Passive calf stretches were performed for 5 × 20 s during 9 weeks by the control group. The intervention group walked backward downhill on the treadmill for 23 min, the speed and the

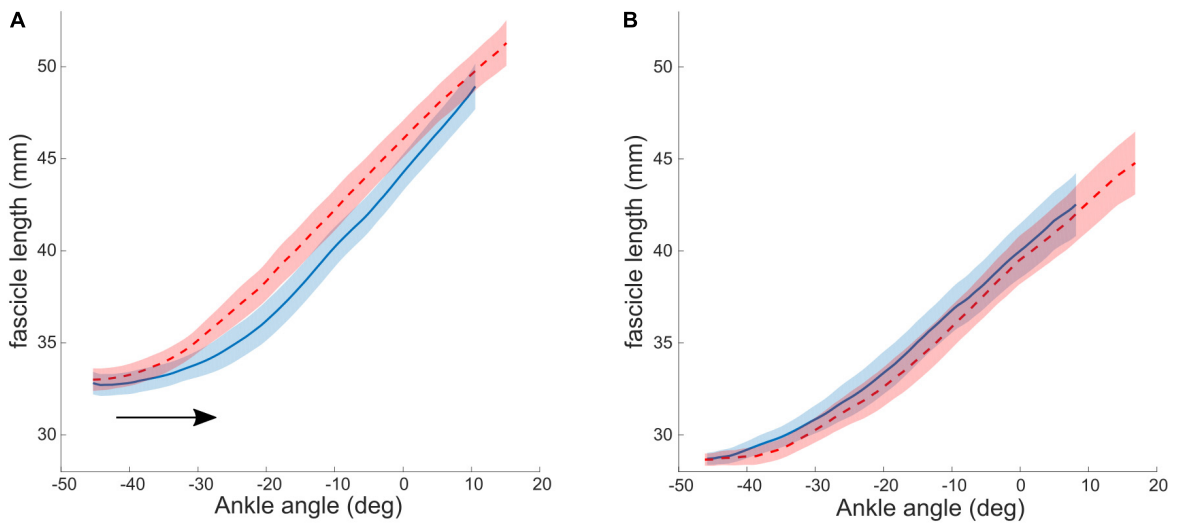

FIGURE 2 | Lengthening profiles of muscle fascicles vs. ankle angle during a passive joint rotation in the intervention **(A)** and control **(B)** group. Negative angles reflect plantarflexion position. The black arrow indicates the shift in ankle angle at which the fascicles start to lengthen. Blue: baseline, red: after 10 weeks of intervention. Values are reported as the median and interquartile range (IQR). Reused from Hösl et al. (2016), held under CC-BY 4.0.

slope of the treadmill were progressively increased and during the last 2 weeks participants had to carry weight belts to increase the load on the calf. No differences were found between the control and the intervention group in terms of muscle morphology or passive ROM. Small improvements were seen after BDTT in ankle dorsiflexion during comfortable walking speed and ambulatory mobility tests which could be a sign of altered neuromuscular control instead of changes in muscle structure.

Difficulties of Muscle Remodeling

The question arising from all these studies is what the signaling pathways are behind the observed changes. Could spastic muscles be stimulated sufficiently to grow even though it has been suggested that growth factors are altered (Pingel et al., 2019), pro-inflammatory cytokines and reduced ribosomal production cause stunted growth (Von Walden et al., 2018) and a reduced skeletal muscle satellite cell number influences muscle morphology after chronic stretch in mice (Kinney et al., 2017). Some have raised the question whether remodeling of muscle might be difficult regardless of the treatment you perform. Nonetheless, promising changes have been observed due to some combination interventions described in this review.

CONCLUSION

Although stretching may be beneficial to prevent worsening of muscle contractures (Wiart et al., 2008), as isolated treatments, they do not promote muscle length growth or improve function in children with CP. Alternative approaches to enhance the effectiveness of stretching exercises are needed. A few promising pathways have been highlighted in this review. Stretching combined with BoNT-A and electrical stimulation appear to have beneficial effects at the joint, but mechanistic work on whether this improved lengthening is provided from muscle or tendon is lacking and warranted. Active stretches (i.e., eccentric contractions) have been proposed as a "stretching alternative" and seem to promote gait and mobility parameters, but improvements in muscle properties are limited. Increasing tendon stiffness to enhance the stretching stimulus "seen" by the muscle seems to be a potentially promising strategy, as Kalkman et al. (2018a) found increases in fascicle lengths after this intervention and Zhao et al. (2011) reported similar results after an active movement and passive stretching intervention. However, more evidence is required to support this notion and additional studies are necessary to investigate the effectiveness of the combined treatments summarized above.

When studying the effect of (stretching) interventions in children with CP, the focus should be on the link between changes in ROM, the associated changes in muscle morphology, and whether these lead to improved muscle and mobility function. A central question that requires answering is: Would an increase in fascicle length lead to improved force production across the newly acquired ROM (Frisk et al., 2019) and does this lead to an improved gait pattern? Another point to consider is that none of the experimental studies discussed here have investigated the long-term effect of their proposed interventions. Since muscle contractures develop over time as children grow, an increase in fascicle length due to in-series sarcomerogenesis might not provide an immediate functional benefit, but it might prevent the development of fixed contractures at later age, thus delaying or possibly making corrective surgeries redundant. Another point to consider is the large inter individual variability in the cause of the increased resistance to stretch in muscles of children with CP. Recent research shows an interdependence between the amount of muscle lengthening and spastic reflexes during stretch (Bar-On et al., 2018). These results warrant individual assessment and treatment planning, taking into account the causes of the reduced ROM in children with CP.

AUTHOR CONTRIBUTIONS

BK, LB-O, TO'B, and CM have all contributed to different aspects in this manuscript.

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REVIEW
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Skeletal Muscle Extracellular Matrix – What Do We Know About Its Composition, Regulation, and Physiological Roles? A Narrative Review

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Skeletal muscle represents the largest body-composition component in humans. In addition to its primary function in the maintenance of upright posture and the production of movement, it also plays important roles in many other physiological processes, including thermogenesis, metabolism and the secretion of peptides for communication with other tissues. Research attempting to unveil these processes has traditionally focused on muscle fibers, i.e., the contractile muscle cells. However, it is a frequently overlooked fact that muscle fibers reside in a three-dimensional scaffolding that consists of various collagens, glycoproteins, proteoglycans, and elastin, and is commonly referred to as extracellular matrix (ECM). While initially believed to be relatively inert, current research reveals the involvement of ECM cells in numerous important physiological processes. In interaction with other cells, such as fibroblasts or cells of the immune system, the ECM regulates muscle development, growth and repair and is essential for effective muscle contraction and force transmission. Since muscle ECM is highly malleable, its texture and, consequently, physiological roles may be affected by physical training and disuse, aging or various diseases, such as diabetes. With the aim to stimulate increased efforts to study this still poorly understood tissue, this narrative review summarizes the current body of knowledge on (i) the composition and structure of the ECM, (ii) molecular pathways involved in ECM remodeling, (iii) the physiological roles of muscle ECM, (iv) dysregulations of ECM with aging and disease as well as (v) the adaptations of muscle ECM to training and disuse.

Keywords: muscle remodeling, matrix metallopeptidase, exercise training, aging, diabetes, fibrosis, connective tissue, gene expression

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INTRODUCTION

Skeletal muscle is an important body-composition component in humans, typically accounting for more than 40% and 30% of total body mass in men and women, respectively (Kim et al., 2002). The most apparent function of skeletal muscles is to generate the forces required to maintain an upright posture and produce movement. However, skeletal muscles do also play important roles in

many other physiological processes, including thermogenesis (Rowland et al., 2015), metabolism (Baskin et al., 2015) and the secretion of numerous peptides for communication with other tissues (Pedersen and Febbraio, 2012). Thus, the promotion and maintenance of skeletal muscle health is of vital importance. Although, in recent years, pharmacological exercise mimetics have attracted increasing scientific interest (Fan and Evans, 2017), it is still physical exercise that is considered the by far most potent and universally applicable tool for these purposes.

Over the past decades, thousands of training studies have been performed in an attempt to identify the exercise modalities most suited to increase muscle size and improve its functional characteristics in different cohorts (for instance, at the time this manuscript was written, Pubmed yielded more than 24,000 results for the search operators "exercise" and "muscle strength"). The outcomes of these studies have inspired various exercise prescription guidelines, probably the best known of which are the position stands published and updated in irregular intervals by the American College of Sports Medicine (2009), Garber et al. (2011). Most studies base their evaluation of the efficacy of training interventions on the examination of contractile muscle cells. Frequently studied parameters involve muscle size as measured at the organ (Fisher et al., 2011) or cellular level (Schoenfeld, 2010), fiber type distribution (Adams et al., 1993), architecture (Aagaard et al., 2001) as well as neural drive to muscles (Folland and Williams, 2007).

The wealth of information on the malleability of skeletal muscles notwithstanding, it is a frequently overlooked fact that muscle fibers are embedded into an extracellular matrix (ECM) consisting of a mesh of collagenous components as well as a mixture of further macromolecules, such as various glycoproteins and proteoglycans. Recent research has demonstrated that the ECM plays an important role in the development (Thorsteinsdóttir et al., 2011), growth (Fry et al., 2017) and repair of muscles (Calve et al., 2010) as well as the transmission of contractile force (Street, 1983). While evidence to demonstrate the malleability of the ECM exists, only a paucity of studies has reported its reactions to different forms of training, suggesting that the physiological role of the ECM is not yet fully appreciated by exercise specialists. Aiming to stimulate further research into the training responses of the non-contractile components of skeletal muscles, we provide an overview over the current state of knowledge concerning the composition, structure and regulation of the ECM, its physiological roles, dysregulations associated with aging and metabolic disorders as well as adaptations to physical exercise.

COMPOSITION AND STRUCTURE OF SKELETAL MUSCLE ECM

The ECM of skeletal muscles is a complex meshwork consisting of collagens, glycoproteins, proteoglycans, and elastin (Takala and Virtanen, 2000; Halper and Kjaer, 2014). Collagens form a network of intramuscular connective tissue (IMCT), i.e., the central, fibrous components of the ECM. The IMCT is typically depicted to be organized in three layers: (i) the endomysium, representing the innermost layer that encloses individual muscle fibers, (ii) the perimysium bundling groups of muscle fibers, and (iii) the epimysium enveloping the entire muscle. The great structural complexity of the IMCT network evidenced by scanning electron micrographs suggests that this traditional classification may be simplistic and that a higher order organization of muscle ECM yet needs to be defined (Gillies and Lieber, 2011). Research into fascial tissues further considers the layers of IMCT as part of a complex system of interconnected and interwoven connective tissues that *"surrounds, interweaves between, and interpenetrates all organs, muscles, bones and nerve fibers, endowing the body with a functional structure, and providing an environment that enables all body systems to operate in an integrated manner"* (Adstrum et al., 2017; Stecco et al., 2018). This system, which is commonly referred to as fascial system, is increasingly recognized as important target in sports medicine (Zügel et al., 2018).

The IMCT contains various forms of collagens with types I and III being most abundant (Duance et al., 1977; Light and Champion, 1984; Gillies and Lieber, 2011; McKee et al., 2019). The endomysium interfaces with the myofiber sarcolemma at a specialized basement membrane, which consists primarily of type IV collagen and laminin (Sanes, 1982; Martin and Timpl, 1987; Kjaer, 2004). The concentration of these two components has been found to differ in dependency of muscle fiber type, with slow twitch fibers featuring substantially greater concentrations of collagen IV but lower concentrations of laminin (Kovanen et al., 1988). Laminin, in turn, serves as ligand for two sarcolemmal receptors – the dystrophin-associated glycoprotein complex and the α7β1 integrin (Grounds et al., 2005) – located at costameres, which are membrane-bound protein structures aligned in register with the Z-disks of myofibrils. Integrins are thought to act in a bidirectional manner, allowing intracellular signaling molecules to regulate external adhesion ("Inside-Out" signaling), and transferring external stimuli to affect cellular processes ("Outside-In" signaling) (Boppart and Mahmassani, 2019). Thereby, cytoskeletal sheer stress induces the intracellular binding of proteins such as talin, vinculin or kindlin, leading to a conformation change of the integrin receptor and allowing the extracellular domains of the receptor to extend toward proteins within the ECM. In addition, integrin ligands from the extracellular space such as laminin, collagen or fibronectin facilitate the formation of a high-affinity upright state, leading to increased binding to ECM proteins and to integrin clustering especially along focal adhesion complexes (Boppart and Mahmassani, 2019). The dystrophin-associated glycoprotein complex is another important factor in providing a mechanical linkage between the contractile components of skeletal muscle (i.e., actin) and the interconnected layers of the IMCT (Ervasti, 1993; Peter et al., 2011). The main components linking the contractile elements of the muscle to the interstitial matrix are shown in **Figure 1**.

The collagen superfamily contains a total of 28 different members, of which types I, III, IV, V, VI, XII, XIII, XIV, XV, XVIII, and XXII have been shown to be present in mature skeletal muscle at the gene and/or protein level (**Table 1**). The fibril-forming types I and III are by far

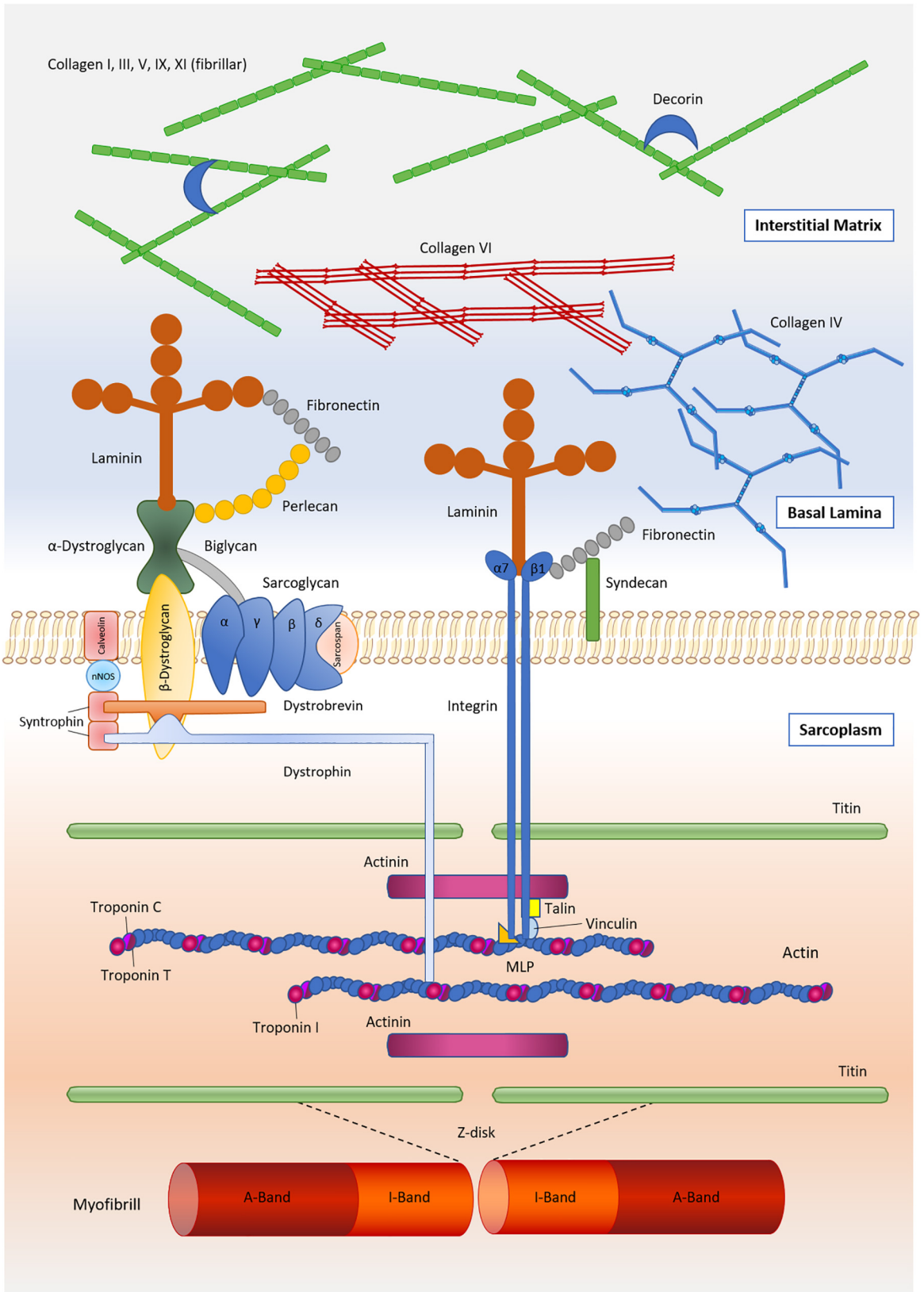

FIGURE 1 | Main components of the skeletal muscle extracellular matrix and its linkage to the contractile components of muscle.

TABLE 1 | Overview of collagenous components of the skeletal muscle extracellular matrix.

Gene(s)	Skeletal muscle RNA expression (protein expression myocytes)*	Protein	Form	Appearance	Role	Source
COL1A1-2	1: 11.0 NX (not detected) 2: 12.1 NX (low)	Collagen type I alpha 1 and 2 chains	Fibrils	Endo-, peri-, and epimysium	Forms strong parallel fibers, confers tensile strength and rigidity	Kovanen (2002)
COL3A1	14.5 NX (not detected)	Collagen type III alpha 1 chain	Fibrils	Endo- and perimysium, myotendinous junction	Forms a loose meshwork of fibers, confers compliance	Kovanen (2002)
COL4A1-6	1: 24.6 NX (not detected) 2: 26.3 NX (high) 3: 17.8 NX (not detected) 4: 7.5 NX (no data) 5: 4.8 NX (no data) 6: 1.0 NX (no data)	Collagen type IV alpha 1-6 chains	Helices	Basal lamina	Produces a network structure, constitutes the basis of the basal lamina	Sanes (2003)
COL5A1-3	1: 5.0 NX (not detected) 2: 3.7 NX (no data) 3: 15.4 NX (medium)	Collagen type V alpha 1-3 chains	Fibrils	Endomysium	Control of collagen fibrillogenesis	Kovanen (2002)
COL6A1-6	1: 20.0 NX (not detected) 2: 30.6 NX (not detected) 3: 31.8 NX (not detected) 4: no data 5: 0.2 NX (low) 6: 0.0 NX (medium)	Collagen type VI alpha 1-6 chains	Beaded filaments	Endo-, peri-, and epimysium (α6-chain) Basal lamina (α3-chain) Myotendinous junction (α5-chain)	Interacts with a large number of molecules and cell surface receptors, maintains muscle functional integrity. Mutations associated with fibrosis and Ullrich, Bethlem or Myosclerosis myopathies	Bönnemann (2011), Sabatelli et al. (2012), Cescon et al. (2015)
COL12A1	21.6 NX (medium)	Collagen type XII alpha 1 chain	FACIT	Endo- and perimysium, myotendinous junction	Linkage protein between fibrillar collagens and other ECM components	Jakobsen et al. (2017)
COL13A1	2.7 NX (not detected)	Collagen type XIII alpha 1 chain	MACIT	Neuromuscular junction	Regulation and formation of neuromuscular synapse. Lack associated with myasthenia	Härönen et al. (2017), Heikkinen et al. (2019)
COL14A1	7.6 NX (not detected)	Collagen type XIV alpha 1 chain	FACIT	Endo- and perimysium, myotendinous junction	Linkage protein between fibrillar collagens and other ECM components. Increases following training at myotendinous junction (protection against strain injury?)	Jakobsen et al. (2017)
COL15A1	12.8 NX (low to medium)	Collagen type XV alpha 1 chain	Multiplexin	Basement membrane	Stabilizes muscle cells and microvessels. Guides motor axons toward muscle targets. Deficiency increases vulnerability to exercise-induced muscle injury and leads to mild forms of myopathies	Eklund et al. (2001), Guillon et al. (2016)
COL18A1	8.1 NX (not detected)	Collagen type XVIII alpha 1 chain	Multiplexin	Basement membrane	May bind growth factors. May link the basement membrane to other basement membrane glycoproteins and endomysium	Gillies and Lieber (2011), Heljasvaara et al. (2017)
COL19A1	3.7 NX (low)	Collagen type XIX alpha 1 chain	FACIT	Basement membrane	Presence at early embryonic stages is relevant for the muscle tissue differentiation. Acts as a cross-bridge between fibrils and other extracellular matrix molecules	Khaleduzzaman et al. (1997), Sumiyoshi et al. (2001)
COL22A1	0.5 NX (not detected)	Collagen type XXII alpha 1 chain	FACIT	Myotendinous junction	Integrates ECM and contributes to mechanical stability of the myotendinous junction. Knockdown of COL22A1 results in dystrophy-like muscle phenotype in zebrafish	Koch et al. (2004), Charvet et al. (2013)

**RNA expression summary shows the consensus RNA-data based on normalized expression (NX) data and protein expression comprises profiles using single as well as independent antibodies directed against different, non-overlapping epitopes on the same protein from the Human Protein Atlas (http://www.proteinatlas.org, Uhlen et al., 2010). FACIT, Fibril Associated Collagen with Interrupted Triple Helices; MACIT: Membrane Associated Collagen with Interrupted Triple Helices. Multiplexin: Collagen with Multiple Triple Helix Interruptions.*

most abundant, with proteomic studies suggesting that they jointly account for approximately 75% of total muscle collagen (McKee et al., 2019). The strong parallel fibers of type I collagen, which are present in the endo-, peri-, and epimysium, are assumed to confer tensile strength and rigidity to the muscle, whereas type III collagen forms a loose meshwork of fibers that bestows elasticity to the endo- and perimysium (Kovanen, 2002). Collagen type IV, a helical molecule, produces a network structure that constitutes the basis of the basal lamina (Sanes, 2003). Collagen type VI has been detected in the epimysial, perimysial, and endomysial interstitium, but in particular in the neighborhood of the basement membrane, where it interacts with the carboxyl-terminal globular domain of type IV collagen (Kuo et al., 1997). Interestingly, collagen VI possesses untypical non-collagenous regions forming a distinct microfibrillar network in most connective tissues (Maaß et al., 2016). Collagen VI mutations result in disorders with combined muscle and connective tissue involvement, including Ullrich congenital muscular dystrophy, Bethlem myopathy, the autosomal dominant limb-girdle muscular dystrophy and the autosomal recessive myosclerosis (Bushby et al., 2014).

Collagen types XII, XIV, XIX, and XXII belong to the Fibril Associated Collagens with Interrupted Triple helices (FACIT; Chiquet et al., 2014; Calvo et al., 2020), whereby collagen type XXII seems to be expressed exclusively at tissue junctions such as the myotendinous junction in skeletal and heart muscle (Koch et al., 2004).

Bioinformatic tools to screen the human proteome of normal and diseased tissues allowed to characterize the global composition of the ECM proteome, or "matrisome." In total, 1,027 genes have been linked to the ECM, whereby core matrisome proteins (ECM glycoproteins, collagens, and proteoglycans) could be distinguished from matrisome-associated proteins (ECM-affiliated proteins, ECM regulators, and secreted factors that may interact with core ECM proteins) (Naba et al., 2016). Given the complexity of human skeletal muscle tissue involving multinucleated muscle fibers, immune cells, endothelial cells, muscle stem cells, non-myogenic mesenchymal progenitors, and other mononuclear cell (Bentzinger et al., 2013a), future research would be needed to elucidate the contribution of each of these cells to the structure and remodeling of the IMCT. Gene signatures derived, e.g., from RNA-seq of isolated muscle fibers and other cell types comprise a promising tool in the deconvolution of bulk skeletal muscle tissue (Rubenstein et al., 2020).

PHYSIOLOGICAL REGULATION OF ECM GENES

The homeostasis of the ECM is maintained through finely tuned anabolic and catabolic processes that are governed by various growth factors, proteoglycans and enzymes responsible for collagen degradation. After binding to membrane-bound receptors, growth factors belonging to the transforming growth factor beta (TGF-β) superfamily have been found to induce the phosphorylation of Smad proteins that transduce extracellular signals to the nucleus where they activate downstream gene transcription resulting in collagen production (MacDonald and Cohn, 2012). Another, albeit less described, factor of similar function is the connective tissue growth factor (CTGF), overexpression of which has been reported to provoke dystrophy-like muscle fibrosis and functional deficits (Morales et al., 2011).

The function of these anabolic factors is mostly regulated by small leucine-rich proteoglycans (SLRPs). Decorin, the prototype member of this family, deactivates the profibrotic TGF-β and CTGF (Zhu et al., 2007; Brandan and Gutierrez, 2013) and also limits fibrillogenesis by directly binding to type I collagen (Reese et al., 2013). Another SLRP is represented by biglycan, which competes with decorin for the same binding site on collagen (Schönherr et al., 1995) and is likely to play a role in both muscle formation and regeneration (Brandan et al., 2008).

Transcriptional regulation of protein formation seems to be an important factor in ECM plasticity. In this respect, it has been shown that protein expression in skeletal muscle is weakly regulated at the mRNA level leading to big differences in mRNA and protein abundance in various tissues (Wang et al., 2019). Interestingly, the pattern of protein regulation depends on protein function, whereby the association between mRNA and protein is higher for ECM and collagen fibril organization (Makhnovskii et al., 2020). Another interesting aspect in the regulation of the amount of ECM proteins is the fact that induction of transcription seems to be rather slow for collagen as it takes almost 3 days to fully induce transcription. In contrast secretion rates are adapted quickly as they are elevated in less than 1 h. In high collagen producing cells, the pathway is controlled by post-transcriptional regulation which requires feedback control between secretion and translation rates (reviewed in Schwarz, 2015).

With respect to tissue remodeling, two families of enzymes, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), are involved in the regulation of ECM homeostasis. MMPs are proteolytic enzymes that degrade various types of collagens and are inhibited by TIMPs (Visse and Nagase, 2003; Alameddine, 2012). Specifically, MMP-1 and MMP-8 initiate the degradation of collagens I and III (prevalent in endo-, peri-, and epimysium), whereas MMP-2 and MMP-9 break down type IV collagen (the major collagenous component of the basement membrane) (Corcoran et al., 1996). TIMP-1, -2, and -4 are capable of inhibiting all known MMPs (Christensen and Purslow, 2016).

ECM AND SKELETAL MUSCLE FORCE

The interaction of actin and myosin as well as many other sarcomeric proteins results in shortening of muscle fibers. Traditional biomechanical models often depict muscle-tendon units as systems, in which the forces generated through fiber shortening are transmitted longitudinally along the muscle fiber and further, at the myotendinous junction, onto the tendon. Close to the myotendinous junction, myofibers feature finger-like processes, which are made from invaginations of the plasma membrane (Knudsen et al., 2015). This structure increases the

surface area available for force transmission. Force transmission is expected to occur between the finger-like processes of the muscle fiber and collagen fibers located within the invaginations through shearing of the basal lamina (Huijing, 1999). The collagens contained here are types XXII, which forms an inner layer, as well as III, VI, XII, and XIV, which lie further away from the muscle fiber membrane (Jakobsen et al., 2017). Although its precise role is still unclear, it is interesting to note that in muscles collagen XXII is exclusively located at the myotendinous junction. In zebra fish, deficiency of collagen XXII has been found to result in muscle dystrophy (Charvet et al., 2013), suggesting that this collagen may serve to maintain the structural integrity and stabilize the myotendinous junction.

Considering the fact that a significant portion of fibers in long muscles terminate intrafascicularly without directly reaching a tendon (Barrett, 1962; Hijikata et al., 1993), however, it is clear that the myotendinous pathway cannot represent the only mechanism of force transfer. Intrafascicularly terminating fibers must rely on a medium arranged in parallel with them to transmit their forces onto the passive components of the locomotor system (Sheard, 2000). As first recognized by Street (1983), it is the network of IMCT within the ECM that facilitates such lateral transfer of contractile force. Force transmission across the IMCT network occurs from contractile proteins across costameres to the endomysium (Bloch and Gonzalez-Serratos, 2003; Peter et al., 2011) – as modeling studies suggest through shearing (Sharafi and Blemker, 2011; Zhang and Gao, 2012) – and further to the perimysium which finally merges with aponeuroses and tendons (Passerieux et al., 2007). The first information about the proportions of longitudinal and lateral force transfer in striated muscle stems from elegant experiments by Huijing et al. (1998). After severing the direct connections of multiple heads of the rat extensor digitorum longus muscle, corresponding to 55% of the total muscle mass, from the joint tendon, Huijing et al. (1998) observed that force was maintained at 84% of that of intact muscle. More recently, Ramaswamy et al. (2011) used a yoke apparatus to directly measure the forces transmitted via the longitudinal and lateral pathway. Their results not only confirmed that more than 50% of force was transmitted laterally but also showed that lateral force transfer was significantly reduced in both dystrophic and old rodents. Their results were later confirmed by Zhang and Gao (2014).

Several arguments suggest that the lateral transmission of force is a biomechanical necessity to maintain muscle integrity and improve contraction efficiency. First, it helps to distribute contractile forces over the entire surface of myofibers, which reduces mechanical stress and protects fibers from overextension. This may be particularly important in fiber end regions, which are usually tapered and therefore ill-suited to tolerate excessive forces (Monti et al., 1999). Indirect support for this hypothesis is provided by studies in older subjects (Hughes et al., 2016) or patients suffering from Duchenne dystrophy (Virgilio et al., 2015), in whom dystrophin (i.e., a costameric protein that establishes the mechanical connection between cytoskeleton, sarcolemma and ECM and, thus, facilitates lateral force transmission) is either lost or impaired and the susceptibility for muscle strain injuries is increased.

Also, lateral force transfer is thought to bridge fibers contracting either at different times or to unequal extents (Yucesoy et al., 2006), which would help maintain fiber alignment and, thus, the muscle's structural integrity (Purslow, 2002). Recently, Dieterich et al. (2017) compared the onset of contraction as determined by electromyography and M-mode ultrasound imaging. Counterintuitively, the authors found the motion onset to precede the electromyography signal in ∼20% of trials, which might be explained by lateral force transfer. Indeed, while longitudinal transmission of forces may be delayed by the need to tauten the elastic elements placed in series with the muscle (Nordez et al., 2009), the translaminar shear linkage between muscle fibers and the IMCT network may allow for immediate force transmission. Finally, lateral force transmission provides a mechanism whereby force may still be generated and transmitted from muscle fibers that are interrupted due to microtrauma or during muscle growth (Purslow, 2010).

In addition to its role in the lateral transfer of contractile force, the ECM may also affect muscle fiber shortening. The contractility of myofibers is often assumed to be constrained by the geometry of its constituting sarcomeres: Sarcomere and, thus, fiber shortening stops when *z*-bands come in contact with myosin filaments. However, these ideas consider only the behavior of the sarcomere as an independent actuator. Under *in vivo* conditions, muscle fibers are embedded into the IMCT network which may interfere with fiber shortening. Indeed, the constant volume principle (Baskin and Paolini, 1967) dictates that during shortening muscle fibers must undergo radial expansion, which has long been experimentally confirmed even at the sarcomeric level (Brandt et al., 1967). Novel computational models and *in situ* measurements in frog muscles by Azizi et al. (2017) have demonstrated that muscle shortening is hindered when radial expansion is limited through physical constraints. Hence, changes in the amount and mechanical properties of the IMCT network into which muscle fibers are embedded may directly affect skeletal muscle contractility. Such a scenario may be represented by muscle fibrosis (Gillies et al., 2017).

ECM IN SKELETAL MUSCLE DEVELOPMENT, GROWTH, AND REPAIR

Apart from force transfer, the skeletal muscle ECM fulfills several important functional roles. Apparently, the IMCT network provides mechanical support to muscle fibers as well as the nerves and blood vessels supporting them. Blood capillaries run in the interstices occupied by endomysium, with their number and density being contingent upon muscle fiber size (Janácek et al., 2009). In addition to this most obvious role, the interaction between myoblasts, differentiated muscle fibers and ECM components is of central importance for the embryogenic development, further growth, and repair of muscle tissue.

The cellular source of the collagenous components of muscle ECM are dedicated IMCT fibroblasts, which originate from different embryogenic sources, including the somites (Nowicki et al., 2003), the lateral plate mesoderm (Pearse et al., 2007) and the neural crest cells (Olsson et al., 2001). As they produce

not only fibroblasts but also adipogenic cells, IMCT fibroblasts may be considered as fibroadipogenic progenitors (Uezumi et al., 2010). Recent research has provided evidence that, in addition to these obvious roles, IMCT fibroblasts and the connective tissues produced by them influence both myogenesis (i.e., the formation of muscle progenitors and their differentiation into multinucleate myofibers) and muscle morphogenesis (i.e., the process in which myofibers are assembled into muscles), thus acting as important regulators of muscle development. These complex regulatory processes occurring during embryogenic development are not covered in detail here, but have been extensively reviewed elsewhere (Nassari et al., 2017; Sefton and Kardon, 2019). In brief, the IMCT guides muscle progenitors to their designated target regions, through a combination of attractive (Hepatocyte Growth Factor, Stromal Cell-Derived Factor) and repulsive signals (Ephrin) (Dietrich et al., 1999; Swartz et al., 2001). Through a myriad of transcription factors expressed in IMCT fibroblasts, the IMCT then promotes the proliferation, survival and differentiation of neighboring myoblasts into mature myofibers (Kardon et al., 2003; Hasson et al., 2010; Iwata et al., 2013; Vallecillo-García et al., 2017). Thus, it may be speculated that the IMCT serves as a mesodermal prepattern that controls the sites of myofiber differentiation and, consequently, the ultimate position, size, and shape of muscles.

As post-mitotic tissues, skeletal muscles depend on satellite cells to adapt and regenerate throughout life. These stem cells reside in specialized niches between the sarcolemma of muscle fibers and their encapsulating basement membranes. Satellite cell maintenance, activation and differentiation are governed by complex cascades of transcription factors. For an extensive review of these cellular circuitries, readers are referred to the recent review by Almada and Wagers (2016). Of particular relevance to this manuscript, a growing body of evidence suggests that satellite cell fate is also strongly influenced by the interactions with the ECM niche in which they reside. Indeed, as a dynamic environment, the stem cell niche transmits mechanical and chemical signals that act to protect quiescent stem cells or induce activation, proliferation, and differentiation.

In the quiescent state, satellite cells express the canonical cell regulator paired box protein 7 (PAX7) (Olguin and Olwin, 2004). *In vitro* studies have demonstrated that a greater portion of satellite cells express PAX7 when cultured on matrigel, a mixture of ECM proteins and growth factors (Wilschut et al., 2010; Grefte et al., 2012). Further support for the notion that the ECM is actively involved in the maintenance of satellite cell quiescence comes from reports that satellite cells removed from their niche quickly enter the cell cycle and lose their capacity for myogenic differentiation (Gilbert et al., 2010). Intriguingly, satellite cells appear to also be able to sense and respond to different ECM mechanical properties. In fact, PAX7 expression and satellite cell survival are greater when cultured on hydrogels that mimic the physiological stiffness of muscle (Gilbert et al., 2010). Also, satellite cells cultured on soft hydrogel feature greater functional capacity after transplantation into recipient muscle (Cosgrove et al., 2014).

In addition, ECM components have been shown to influence stem cell division. Specifically, the proteins fibronectin (Bentzinger et al., 2013b) and collagen VI (Urciuolo et al., 2013) as well as the proteoglycans syndecan 3, syndecan 4, perlecan, and decorin (Cornelison et al., 2001; Brack et al., 2008) have been identified as the niche constituents influencing the balance between differentiation and self-renewal and, thus, the maintenance of skeletal muscles' regenerative capacity.

Upon muscle trauma or in response to increased loading, the usually mostly quiescent satellite cells become activated and differentiate into myoblasts to finally fuse into mature myofibers. While this process requires the timely expression of various transcription factors, such as myogenic factor 5, myogenic determination protein or myogenin (Almada and Wagers, 2016), several studies point to the influence of the ECM on each of these steps. Experiments with mouse (Grefte et al., 2012) or porcine myoblasts (Wilschut et al., 2010) have shown that myoblast fusion is positively influenced by matrigel but not by single substrates present in the ECM niche. The contributions of single proteins are still poorly understood, however, the concomitant presence of poly-D-lysine and laminin (Boonen et al., 2009), glycosaminoglycans (Rønning et al., 2013), and heparin sulfate proteoglycans (Gutiérrez and Brandan, 2010) appear to play a prominent role in satellite cell proliferation and differentiation. Upon activation of skeletal muscle stem cells, local remodeling of the ECM is accompanied by the deposition of laminin-α1 and laminin-α5 into the basal lamina of the satellite cell niche (Rayagiri et al., 2018). In mice, it has been shown that muscle satellite cells produce ECM collagens to maintain quiescence in a cell-autonomous manner with collagen V being a critical component of the quiescent niche, as depletion leads to anomalous cell cycle entry and gradual diminution of the stem cell pool (Baghdadi et al., 2018). Just as for the maintenance of quiescence, adequate mechanical properties of the ECM niche may also be important for satellite cell maturation. Indeed, myotubes have been found to differentiate optimally on substrates with muscle-like stiffness (Engler et al., 2004). Jointly, these data suggest that ECM stiffening accompanying both different musculo-skeletal disorders and the aging process may negatively influence a muscle's regenerative capacity.

REMODELING OF MUSCLE ECM WITH AGING

At older age, skeletal muscles typically demonstrate fibrotic morphology (Lieber and Ward, 2013). As opposed to fascial densification, where the general structure of collagens may be preserved (Pavan et al., 2014), age-associated muscle fibrosis is characterized by the loss of the clear two-directional lattice orientation of healthy perimysial collagen fibers and its replacement by an erratic fiber network featuring decreased crimp formation (Järvinen et al., 2002). Also, absolute collagen content and (non-enzymatic) cross-linking of collagen fibers may be increased (Haus et al., 2007b). Thereby, the elastic modulus of the ECM can be increased approximately 35-fold (from ∼12 kPa in young to ∼418 kPa in old muscle; Yin et al., 2013), with this effect being driven by an accumulation of densely packed and extensively cross-linked collagen (Wood et al., 2014). In

large-bodied, long-lived animals, such as the Weddell seals, a 35–40% increase in extracellular space has been observed as total and relative collagen contents increase with age. However, this increase is associated with a shift toward a higher ratio of type I to type III collagen (Hindle et al., 2009). Furthermore, collagen type IV concentration is enhanced in the basal lamina of slow twitch muscles, whereas laminin concentration seems to decrease with age (Kovanen et al., 1988). The increased deposition of basal lamina proteins has also been shown to expel satellite cells from their niches, which affects the regulation of satellite cell divisions (Snow, 1977) and may explain the lower numbers of satellite cells typically counted in old as compared to young muscle (Brack et al., 2007). The loss and functional inactivation of stem cells that negatively affects tissue homeostasis can be considered a general hallmark of aging (López-Otín et al., 2013) that must be considered a universal force driving the aging of muscle (Brack and Muñoz-Cánoves, 2016) and other tissues (Oh et al., 2014). In addition to its effects on satellite cells, a dysregulated basal lamina is also expected to disturb the muscle's regenerative capacity through inadequate support of muscle fibers and disorganized scaffold orientation (Sanes, 2003). A review including an extensive summary of the effects of aging on skeletal muscle ECM has recently been published by Etienne et al. (2020).

Interestingly, data from transcriptional profiling of muscles derived from young and old rats suggest that out of 682 probe sets that differed significantly between young and old animals, 347 genes actually decreased (rather than increased) in aged/sarcopenic muscle relative to young muscles. Of these genes, 24% have been shown to exert a biological role in the ECM and cell adhesion (Pattison et al., 2003). These data support the hypothesis that age-associated changes in the ECM might be driven by a decreased degradation capacity rather than by increased synthesis of collagenous structures. Especially, MMPs seem to play an important role in these processes (de Sousa Neto et al., 2018). This is further supported by findings that suggest a diminished resistance exercise-induced remodeling capacity of ECM structures in aged muscles (Wessner et al., 2019). While the mechanisms are not yet fully understood, these changes are also believed to directly impair muscle function by hindering fiber contractility (Azizi et al., 2017) and lateral force transmission (Sharafi and Blemker, 2011).

DYSREGULATION OF SKELETAL MUSCLE ECM CONSEQUENT TO METABOLIC DISORDERS

It is well known that skeletal muscle plays an important role for the insulin-stimulated uptake of glucose (Richter and Hargreaves, 2013). The role of the ECM in this context might be less clear. Muscle-specific integrin β1-deficient mice exhibit a reduction of the insulin-stimulated glucose infusion rate and glucose clearance despite no alterations in food intake, weight, fasting glucose, insulin levels, and GLUT4 protein expression (Zong et al., 2009) suggesting a relationship between aberrant integrin signaling and the development of type 2 diabetes. Furthermore, it has been shown in an animal model of diabetes that impaired insulin sensitivity is associated with reduced protein levels of the Dp427 isoform of dystrophin and the alpha/beta-dystroglycan subcomplex (Mulvey et al., 2005).

Increased amounts of type I and III collagen were found in both type 2 diabetic and also non-diabetic obese subjects (Berria et al., 2006) and overfeeding in humans was associated with increases in the expression of genes associated with the IMCT (collagens I, III, IV, V, SPARC, integrin; Tam et al., 2014) and alterations in gene pathways related to ECM receptor interaction, focal adhesion, and adherens junction (Tam et al., 2017). However, feeding a high-fat diet to mice led to a reduction of COL1, COL3, and COL6 gene expression levels, but not protein levels (Tam et al., 2015).

The degradation of collagens through MMPs has been shown to be an essential constituent of ECM remodeling (Cui et al., 2017). Whether this might also be true in the context of diabetes has been investigated in an animal study. Interestingly, the genetic depletion of MMP9 did not induce insulin resistance in lean mice despite resulting in an increase of collagen IV. However, when mice were fed a high-fat diet the deletion caused a profound state of insulin resistance. These results further strengthen the role of IMCT components in the progress of muscle insulin resistance, especially in a state of overfeeding (Kang et al., 2014).

Finally, hyaluronan, a major constituent of the ECM is increased in high-fat diet-induced obesity in mice. Treatments with PEGPH20, which dose-dependently reduces hyaluronan in muscle ECM is suggested for the treatment of insulin-resistance with a concomitant decrease in fat mass, adipocyte size, as well as hepatic and muscle insulin resistance (Kang et al., 2013).

To summarize, various components of the ECM have been shown to be affected in various stages of diabetes. Studies on whether diabetes is linked to muscle weakness are controversial (Leong et al., 2015; Li et al., 2016) and it remains to be elucidated whether the changes in ECM-related pathways are directly involved in this context.

ADAPTATIONS TO PHYSICAL TRAINING AND DISUSE

The first evidence to indicate the malleability of IMCT in response to physical activity was published as early as in the 1970s, when Suominen and Heikkinen (1975) and Suominen et al. (1977) found greater levels of prolyl hydroxylase (an enzyme promoting the biosynthesis of collagen) in endurance-trained athletes as well as, in a longitudinal study, after eight weeks of aerobic training. The effect of endurance exercise on the pro-collagenous enzymatic activity was later found to be more prominent in red as compared to white muscle (Takala et al., 1983). Direct measurements of collagen content first performed in the late 1980s confirmed that the (type IV) collagen content increased in the fatigue-resistant soleus muscle of rats following lifelong endurance training (Kovanen et al., 1988). The exercise-induced increase in collagen notwithstanding, Gosselin et al. (1998) found that the muscle stiffening observed

with advancing age could be countered by an endurance exercise intervention, which the authors related to reduced hydroxylysylpyridinoline cross-linking of collagen fibers.

The effects of immobilization on the skeletal muscle ECM are not entirely unequivocal. Early studies by Karpakka et al. (1990, 1991) found both hydroxylase activity and hydroxyproline (an amino acid constituting collagens) content to be reduced in rats. Changes in collagen content in response to short-term immobilization or disuse were later found to be rather small (Savolainen et al., 1988; Haus et al., 2007a), which may be explained by a relatively slow turnover rate. A more recent study, by contrast, found the content of collagen I and the biomechanical properties (elastic modulus, max stress and yield stress) of crural fascia ensheathing the rat triceps surae muscle to be significantly increased after as little as 21 days of hindlimb unloading (Huang et al., 2018). Interestingly, these changes could be prevented through the application of vibration to the rats' hind paws twice a day. In non-exercising humans, immunohistochemical staining suggested no changes in the density of the collagen I network after 60 days of bed rest. In subjects performing a countermeasure exercise protocol consisting of reactive jumps on a sledge system, by contrast, collagen I immunoreactivity was reduced as compared to baseline levels (Schoenrock et al., 2018).

Yet another model that allows for the adaptability of muscles' ECM to be studied is functional overload induced by surgical synergist elimination. In one of the first respective studies, Williams and Goldspink (1981) severed the tendons of the plantaris and gastrocnemius muscles of male rats to overload the soleus muscles. The muscle hypertrophy observed three weeks after tenotomy was accompanied by increases in the IMCT concentration (>45%) and the IMCT-to-muscle tissue ratio. Histological analyses further suggested that the increase in IMCT was mostly due to a thickening of the endomysium. Focusing on the myotendinous junction, Zamora and Marini (1988) performed similar experiments and isolated the rat plantaris muscle through tenotomy of the soleus and ablation of the gastrocnemius muscles. In comparison with control animals, the fibroblasts located at the myotendinous junction developed a higher degree of activation of cytoplasm, nucleus and nucleolus after as little as one to two weeks of functional overload. A more recent study tested the effect of IL-6 on overload-induced ECM remodeling by comparing wild-type and IL-6-knockout mice (White et al., 2009). While the gains in myofiber cross-sectional area were similar after 21 days of functional overload, the increases in muscle wet weight were significantly larger in IL-6-knockout mice. Histological analyses confirmed that this surplus gain in muscle weight could be explained by significantly larger increases in non-contractile tissue content and hydroxyproline concentration, which is indicative of collagen content and fibrosis. In agreement with this observation, procollagen-1, IGF-1, and TGF-β mRNA levels were significantly higher in IL-6-deficient mice. Conversely, mRNA expression of MyoD, a transcription factor required for myo- rather than fibrogenic differentiation of satellite cells (Zammit, 2017), was significantly attenuated in animals lacking IL-6. Jointly, these results indicate that synergist elimination induces an increase in IMCT content and, specifically, a thickening of endomysial structures in overloaded muscles. These adaptations may serve to modulate the muscles' non-contractile structures to increased functional demands. IGF-1 appears to play an important role in the regulation of this process, as lack of IGF-1 has been shown to lead to excessive accumulation of IMCT and, potentially, impaired muscle regenerative potential.

One of the first studies to test and compare different forms of resistance-like exercise in men was performed by Brown et al. (1999) who reported that, following a single bout of concentric contractions, markers of collagen breakdown (hydroxyproline and serum type I collagen) were not increased. By contrast, eccentric muscle action increased serum collagen levels by >40% for up to 9 days post-exercise, indicating that eccentric contractions may be superior in promoting collagen breakdown. These results were confirmed in two later studies similarly using high-intensity eccentric exercise that found both increased procollagen processing and type IV collagen content as well as higher MMP and TIMP activities (Crameri et al., 2004; Mackey et al., 2004). Interestingly, Crameri et al. (2004, 2007) also reported an increase in tenascin C, a glycoprotein present in the ECM that is assumed to direct cell migration following injury, irrespective of whether muscle damage was induced by voluntary or electrically induced muscle damage. The transient upregulation of tenascin C and other ECM glycoproteins (e.g., fibronectin and hyaluronic acid) is usually referred to as the "transient matrix," the appearance of which is considered an essential first step for successful muscle repair, as it provides important cues driving muscle stem cell regenerative potential (Calve et al., 2010; Tierney et al., 2016). The release of ECM glycoproteins is reportedly accompanied by increased MMP-9 activity in young, but decreased MMP-9 and MMP-15 activity in old subjects (Wessner et al., 2019). These findings suggest that an acute bout of resistance exercise triggers a catabolic response in young muscle but that this effect may be impaired at older age. The subsequent anabolic reaction, characterized by the upregulation of structural collagens (I, III, IV) and laminin, has been found to occur with a significant delay, thus suggesting that muscle repair consequent to an acute bout of damaging (lengthening) contractions follows a biphasic nature (Mackey et al., 2011; Hyldahl et al., 2015). Interestingly, a recent study by Sorensen et al. (2018) found that the appearance of the transient matrix was blunted in physically active old as compared to young subjects. This observation supports the notion that dysregulated ECM cues may be responsible for the increased ECM deposition and reduced stem cell activity typically seen in older muscle (Grounds, 1998).

One of the first studies to directly compare different forms of muscular contraction in terms of their acute ECM remodeling potential was published by Heinemeier et al. (2007). These authors performed a study in rodents and found that the activity of genes associated with collagen biosynthesis (e.g., collagens I and III) as well as growth factors (e.g., TGF-β1) were upregulated after all forms of physical exercise but most prominently so after eccentric training. In humans, collagen protein fractional synthesis rates have also been proposed to be more increased following an acute bout of eccentric as compared

to concentric training (Holm et al., 2017), although this notion is not unchallenged (Moore et al., 2005). Jointly, these results suggest that particularly eccentric exercise is a potent stimulus that induces microtrauma and IMCT cell turnover, with the latter assumed to represent the organism's attempt to prevent the muscle from re-injury (Mackey et al., 2011; Hyldahl et al., 2015; Takagi et al., 2016). In fact, diminished MMP activity after prolonged training consisting of electrically evoked isometric contractions in rats may reflect successful ECM reinforcement (Ogasawara et al., 2014), whereas prolonged increases in MMP- and TIMP-activity in the plantaris muscle of mice following surgical removal of the gastrocnemius and soleus muscle could be indicative of ongoing ECM remodeling (Mendias et al., 2017).

In addition to contraction mode, skeletal muscle ECM may also be sensitive to exercise intensity. Carmeli et al. (2005) tested the effects of treadmill running at either high or low intensity in rats and found that MMP-2 (one of the enzymes responsible for the breakdown of collagen IV mainly present in the muscle's basement membrane) was increased after high-intensity exercise only. In humans, by contrast, one study by Holm et al. (2010) compared the effects of unilateral knee extension exercise as performed at either low or high (16% or 70% of the individual one-repetition maximum, respectively) intensity, with the number of repetitions adjusted to match the interventions for the total load lifted. In this study, collagen fractional synthesis rates were evenly increased following both interventions.

In terms of ECM adaptations to prolonged resistance training, only data from animal studies exist. de Sousa Neto et al. (2018) reported that 12 weeks of resistance training consisting of ladder climbs with progressive, additional loads equivalent to 65–100% of each individual's maximum carrying capacity upregulated MMP-2 activity in the plantarflexor muscles of old rats, while down-regulating MMP-2 and MMP-9 in blood circulation. The authors' conclusion that resistance training might, therefore, be a useful tool to maintain ECM remodeling at older age has recently received empirical support by another training study in rats that used the same training protocol and showed a reduced deposition of connective tissue in trained older muscles (Guzzoni et al., 2018).

To summarize, several studies investigating the acute effects of physical activity in both rodents and men have indicated that exercise may stimulate both the degradation and synthesis of collagen in skeletal muscle. The repair of exercise-induced microtrauma follows a biphasic pattern, in which glycoproteins first create a transitional matrix to guide catabolic processes, and anabolic processes to reinforce the IMCT structure occur with a significant delay. The potential of exercise to induce ECM remodeling seems to be dependent on contraction mode with eccentric contractions triggering a greater response than either concentric or isometric muscle action. Few studies testing the results of different exercise intensities are available, with so-far results suggesting that protein breakdown (but not synthesis) may be provoked more strongly by higher intensities. Disuse acutely downregulates the activity of enzymes related to the biosynthesis of collagens, although at the protein-level changes occur at a slow rate. Cross-sectional comparisons involving (mostly endurance-) trained rodents suggest that chronic physical activity may result in a reinforced IMCT phenotype. The only long-term longitudinal training studies available to date have been performed in rodents and suggest that prolonged resistance training may be useful in countering excessive IMCT accumulation at older age. The physiological and functional consequences of training-induced IMCT remodeling require further investigation.

CONCLUSION

The present review aimed to provide an overview over the current state of knowledge concerning the skeletal muscle ECM, which plays an essential, albeit frequently underestimated role in the maintenance of muscle homeostasis, influences muscle function and adaptation and may be a key for the treatment of muscular and metabolic disorders consequent to aging or disease.

As a complex meshwork of various collagens, glycoproteins, proteoglycans and elastin, the ECM embeds contractile muscle fibers and serves via integrins and the dystrophin-associated glycoprotein complex, respectively, as biochemical and mechanical interface between muscle cells and their surroundings. The assembly of its collagenous scaffold is mostly promoted by the growth factors TGF-β and CTGF, which are regulated by different proteoglycans, such as decorin and biglycan. Moreover, proteolytic enzymes (MMPs) as well as their inhibitors (TIMPs) are involved in ECM regulation.

Functionally, the ECM serves as medium for the transmission of contractile force, which may not only serve to increase the efficiency of muscular contraction but also to protect muscle fibers from excessive stress and facilitate healing of microtrauma. In addition to its functional role, the ECM is actively involved in the regulation of the muscle's pool of satellite cells. ECM niches, established between sarcolemma and basement membrane, protect satellite cell from entering the cell cycle and, thus, help maintain the muscle's regenerative potential. Specific ECM components, such as fibronectin, collagen VI and different proteoglycans, may additionally promote stem cell division. Conversely, laminin, glycosaminoglycans and other proteoglycans have been shown to promote satellite cell differentiation and their fusion into mature myofibers.

Scientific evidence further demonstrates that the ECM of skeletal muscles is a malleable tissue that may undergo remodeling processes consequent to aging, disease, physical training or disuse. Specifically, aging typically leads to overall increased deposition of collagenous tissue, changes in collagen composition (shift toward higher type I to type III collagen) and increased non-enzymatic collagen cross-linking (through advanced glycation end products). These changes, which are possibly mediated through decreased MMP activity, lead to stiffening of the muscle's ECM and may impair the muscle's function and regenerative potential.

Extracellular matrix remodeling may also be associated with metabolic disorders, such as diabetes. Excessive food intake has been found to lead to increased expression of ECM-related genes (collagens I, III, IV, V, SPARC, integrin). In turn, such remodeling

may impair integrin signaling, thus reducing insulin sensitivity. Further ECM components potentially representing targets for insulin resistance are hyaluronan, the dystrophin-dystroglycan complex as well as MMP9.

Finally, ECM remodeling may be triggered by physical exercise. While actual training studies are scant, there is evidence to show that exercise may acutely promote both increased collagen synthesis (collagens I, III, TGF-β1) and degradation (MMP2, MMP9). Cross-sectional studies in humans and longitudinal studies in rodents further suggest that such increased collagen turnover may lead to reinforced collagenous structures in chronically trained subjects and prevent excessive collagen deposition (i.e., fibrosis) in elderly muscle. Studies investigating the consequences of prolonged disuse have shown controversial results. While early studies reported decreased hydroxylase activity and hydroxyproline content after short-term immobilization, more recent works found increased collagen I content after 21 days of hindlimb unloading in rats but no change after 60 days of bed rest in humans. Further research and particularly human training studies are required to investigate the influence of different training modalities on ECM structure and composition.

AUTHOR CONTRIBUTIONS

RC contributed to the literature research and drafted the manuscript. MG and BW contributed to the literature research and revised the manuscript. All authors have approved the final version of the manuscript and agreed to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Skeletal Muscle Shape Change in Relation to Varying Force Requirements Across Locomotor Conditions

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Contractions of skeletal muscles to generate *in vivo* movement involve dynamic changes in contractile and elastic tissue strains that likely interact to influence the force and work of a muscle. However, studies of the *in vivo* dynamics of skeletal muscle and tendon strains remain largely limited to bipedal animals, and rarely cover the broad spectra of movement requirements met by muscles that operate as motors, struts, or brakes across the various gaits that animals commonly use and conditions they encounter. Using high-speed bi-planar fluoromicrometry, we analyze *in vivo* strains within the rat medial gastrocnemius (MG) across a range of gait and slope conditions. These conditions require changes in muscle force ranging from decline walk (low) to incline gallop (high). Measurements are made from implanted (0.5–0.8 mm) tantalum spheres marking MG mid-belly width, mid-belly thickness, as well as strains of distal fascicles, the muscle belly, and the Achilles tendon. During stance, as the muscle contracts, muscle force increases linearly with respect to gait–slope combinations, and both shortening and lengthening fiber strains increase from approximately 5 to 15% resting length. Contractile change in muscle thickness (thickness strain) decreases (r^2 = 0.86; p = 0.001); whereas, the change in muscle width (width strain) increases (r^2 = 0.88; p = 0.001) and tendon strain increases (r^2 = 0.77; p = 0.015). Our results demonstrate force-dependency of contractile and tendinous tissue strains with compensatory changes in shape for a key locomotor muscle in the hind limb of a small quadruped. These dynamic changes are linked to the ability of a muscle to tune its force and work output as requirements change with locomotor speed and environmental conditions.

Keywords: bulging, locomotion, length change, contraction, compliance, series elastic element

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INTRODUCTION

Skeletal muscle contractions that produce and control *in vivo* movement involve dynamic contractile and elastic tissue strains, as well as changes in muscle shape, that likely interact to influence the force and work production of a muscle. These dynamic changes facilitate the diverse roles of muscles as well as their tendons, which act as biological springs (Roberts and Azizi, 2011).

However, studies of the *in vivo* dynamics of skeletal muscle and tendon strains remain largely limited to humans and other bipedal animals, and have rarely examined the broad range of movement requirements met by muscles that may operate as motors, struts, or brakes across the various gaits animals commonly use and conditions they encounter (Dickinson et al., 2000).

When humans and other animals decelerate or move downhill, the function of limb muscles, as a whole, must shift toward eccentric contractions to absorb energy, braking the body's motion (Gabaldon et al., 2004; Ishikawa and Komi, 2004; Lichtwark and Wilson, 2006; Biewener and Daley, 2007; Konow and Roberts, 2015; Helm et al., 2019). By contrast, when the locomotor task is to accelerate, move uphill, or climb stairs, muscles of the limbs must shift toward concentric contractions to generate greater net positive work (Gillis and Biewener, 2002; Daley and Biewener, 2003; Gabaldon et al., 2004; Hoyt et al., 2005; McGuigan et al., 2009). In comparison, when moving at steady speed on the level, the work requirements of limb muscles are substantially reduced (Cavagna et al., 1964). Indeed, distal limb muscles, for which direct *in vivo* measurements of mechanical work have been obtained, favor more economical force production with limited fascicle strain and work output (Roberts et al., 1997; Biewener and Roberts, 2000). The architecture of these distal muscle–tendon units (MTUs) also favors elastic energy storage and return from their aponeuroses and tendons, with elastic energy savings that substantially reduces muscle work requirements for steady level locomotion (Biewener and Baudinette, 1995; Biewener et al., 1998; McGuigan et al., 2009).

Interactions between a muscle's fascicles and its aponeuroses and free tendon also significantly affect the force and work dynamics of a muscle and the MTU as a whole. When landing or running downhill, elastic energy stored in the tendon is recycled or absorbed by the stretch of a muscle's fascicles (Griffiths, 1991; Konow et al., 2012; Konow and Roberts, 2015). The rapid stretch of the MTU initially accommodated by the stretch of the muscle's tendon provides a "mechanical buffer" that allows the muscle's fascicles to stretch more slowly, reducing the risk of eccentric muscle injury (Griffiths, 1991; Reeves and Narici, 2003; Roberts and Azizi, 2010; Roberts and Konow, 2013). When accelerating or running uphill, elastic energy stored in tendon and aponeuroses can be released more rapidly than the positive shortening work of the muscle's fascicles, increasing MTU power output as a whole (Daley and Biewener, 2003; McGuigan et al., 2009). As observed for the human medial gastrocnemius (MG) (Lichtwark and Wilson, 2006), interactions between the muscle's fascicles and elastic tendon not only allow the muscle's fascicles to perform shortening work during both incline and decline locomotion but also likely allow the fascicles to operate at shortening velocities that maximize their power output. Similarly, during stair ascent and descent human MG fascicles exhibit differing length change behaviors from the MTU, presumably due to tendon compliance (Spanjaard et al., 2007).

In addition to interactions between fascicle and tendon (or aponeurosis) strains of MTUs, skeletal muscles also undergo changes in width and thickness during contractions. Based on *in situ* muscle studies, 3D changes in muscle shape have been hypothesized to result from the interplay of muscle fiber forces and the resistance of connective tissue to fiber rotation and contractile bulging (Azizi et al., 2008; Holt et al., 2016). Specifically, at low muscle forces, fiber shortening was observed to be associated with the rotation of fascicles and an increase in pennation angle, so that the muscle thickness increased. However, at high muscle forces, fibers shortened with less rotation, and muscle shape change was interpreted as favoring an increase in width versus thickness (Holt et al., 2016). Although these prior studies suggest that 3D changes in muscle shape may significantly affect the force and work output of the MTU as a whole, the phenomenon of 3D bulging remains poorly studied *in vivo*.

Measurements of muscle shape change under dynamic locomotor conditions across a range of gait (speed) and slope conditions are therefore needed to better understand how these variables may affect the force and work output of a muscle. Prior ultrasound studies have shown that the human MG resists bulging in thickness (reduced fascicle rotation) during high-force contractions when cycling at high crank torque (Dick and Wakeling, 2017). We hypothesize that a similar trade-off between width and thickness bulging will occur in the rat MG during unrestrained *in vivo* locomotion; specifically, we predict that the rat MG will undergo widthwise bulging during high-force contractions, minimizing fascicle rotation to augment whole muscle force, along with reductions in widthwise bulging, and increases in thickness, to increase whole muscle shortening velocity during low-force contractions. We test this hypothesis by obtaining fluoromicrometry measurements (Konow et al., 2015; Camp et al., 2016) of muscle shape change, fascicle strain, and tendon strain, as well as measurements of force from the rat MG MTU across a comprehensive range of gait and slope conditions.

MATERIALS AND METHODS

Animals, Training, and Surgery

Five adult male Sprague Dawley rats (275–320 g) obtained from Charles River Laboratories (Wilmington, MA, United States) were housed at the Concord Field Station (Harvard University) and studied in compliance with IACUC- and USDA-approved protocols. Rats were trained 5 days per week to locomote on a DC motorized treadmill (10 cm wide × 60 cm long, equipped with a textured rubber belt to prevent slipping) for 2–3 weeks, until they were able to move steadily at all gaits at each particular speed (walk: 0.25 ± 0.02 m s^{-1}; trot: 0.51 ± 0.03 m s^{-1}; gallop: 0.75 ± 0.06 m s^{-1}) and slopes (downhill: $-20°$, level, and uphill: $+20°$). Animals were encouraged to maintain position on the treadmill by gently tapping or briefly gusting their hindquarters with compressed air. The treadmill was customized with a carbon fiber radio-translucent base (Airex C70-40 0.25″ foam core; Dragonplate.com; Elbridge, NY, United States) beneath the rubber treadmill belt to facilitate dorsoventral X-ray imaging (see below). The animals moved within an enclosure constructed of 0.318 mm Plexiglas sidewalls.

Following treadmill training, the rats were anesthetized (isoflurane: 2–4% induction and 1–2% maintenance,

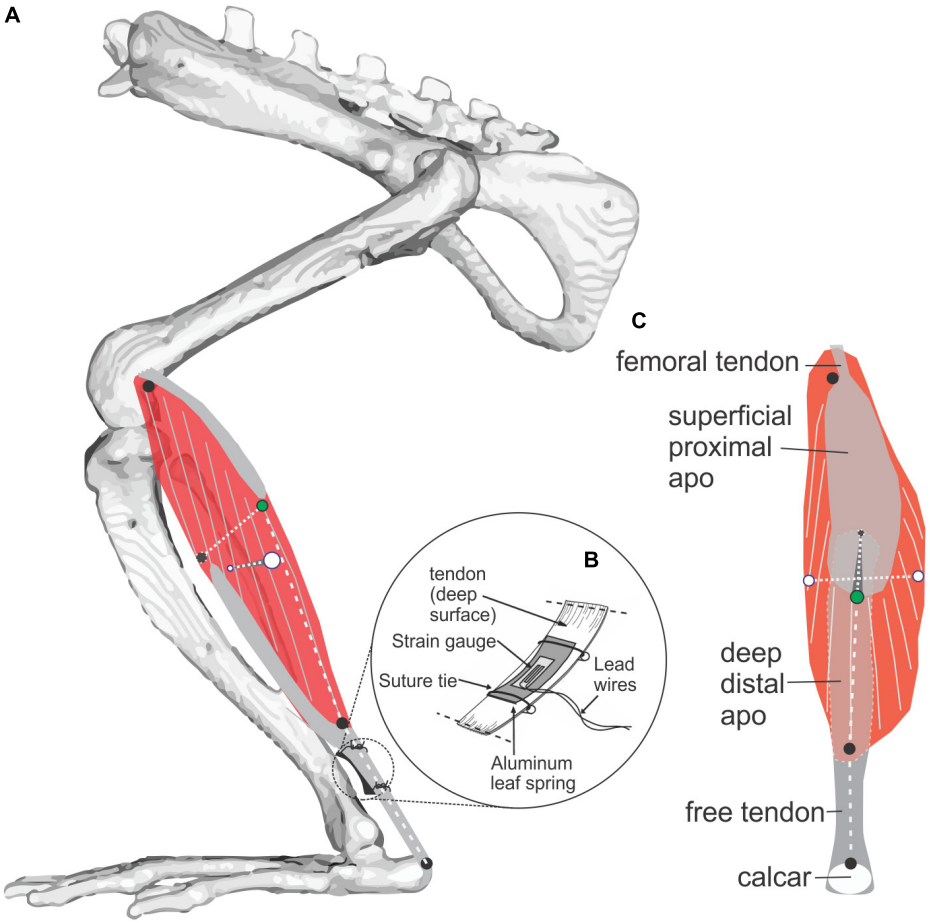

FIGURE 1 | Study muscle and instrumentation. **(A)** Diagram of the rat MG (medial view) showing architecture of muscle fibers (gray on red muscle belly) and aponeuroses (gray). Radio-opaque marker placements at superficial, deep, medial, and lateral muscle surfaces is shown with black and green markers used to measure muscle thickness, and white markers used to measure muscle width. Length of the muscle belly and its tendon were measured using markers along the muscle's line of action at the positions indicated by three black circles. **(B)** Placement of a foil strain-gauge transducer on the muscle's tendon (Richards and Biewener, 2007) to measure MG force (separate experiment; *N* = 3, see **Figure 3**). **(C)** Superficial (posterior) view of the MG showing marker locations.

administered at 0.8–1.0 L O_2 min^{-1} through a small nose cone) and their left hind limb prepped for surgery (fur removed, followed by betadine scrub). Under sterile surgical conditions, the belly of the left MG was exposed by means of a 2–3 cm medial skin incision and blunt dissection of the superficial-most surrounding fascia. Although dissection of the overlying fascia may influence muscle shape change due to fascial interactions (Huijing et al., 2003; Maas and Sandercock, 2010), this is unavoidable to obtain direct recordings of length change using the methods employed here and in normal physiological conditions, as studied here, these effects are believed to be small (Maas and Sandercock, 2008). Seven sterilized radio-opaque tantalum spheres (0.5–0.8 mm; Abbott Balls, West Hartford, CT, United States) were implanted into the muscle using a trochar constructed from 21- or 18-gauge hypodermic needle stock, respectively, equipped with a stainless steel rod plunger. Markers were placed at the following locations: mid-belly lateral, medial, superficial, and deep epimyseal surfaces; distal and proximal aponeuroses, and distal end of the Achilles tendon, at its calcar attachment (**Figure 1**; and see **Supplementary Figure S1** without overlying labels). The proximal origin of the MG was exposed by reflecting the overlying medial hamstring muscles to implant the proximal aponeurosis marker. The rat MG is a unipennate muscle with fascicles running from the superficial proximal aponeurosis to the deep distal aponeurosis. The superficial muscle belly marker positioned at the distal end of the proximal aponeurosis was aligned with the distal MG fascicles that insert on to the deep aponeurosis at the location of the distal aponeurosis marker. These markers, therefore, provided measurements of distal fascicle strains in relation to changes in muscle width, thickness, whole muscle length, and tendon length. We should note that our measurements assume no change in the alignment of the epimysium at these marker locations, and thus the transverse plane of muscle thickness and width, with respect to the axis of overall muscle tension over a contraction cycle across gait–slope conditions. Additionally, recent bi-planar ultrasound measurements of human tibialis anterior 3D shape change indicate that changes in cross-sectional

shape may vary over proximo–distal regions of muscle length during isometric contractions (Raiteri et al., 2016).

After closing the medial skin incision with 4-0 nylon suture (Ethicon, Inc., Somerville, NJ, United States) the rats were allowed 5–7 days to recover with analgesics (Flunixin meglumine, 2 mg/kg) administered every 12 h for the first 2 days to minimize post-operative pain and inflammation.

Fluoromicrometry Recordings

To obtain *in vivo* recordings of muscle–tendon shape and length change, the treadmill was positioned so that the dorsoventral and mediolateral movements of the rat subject (**Figure 2**) could be captured by two orthogonally arranged X-ray C-arms (OEC 9400, Radiological Imaging Services, Hanover, PA, United States) operating at 70–90 kVp and 2.0–3.8 mA, each equipped with a high-speed video camera (Photron PCI-1024; 1024 × 1024 pixels, with 1/2000 s exposure; San Diego, CA, United States). X-ray settings were established to provide the most ideal contrast of the tantalum beads with respect to surrounding musculoskeletal structures. Walking was captured at 125 frames s^{-1}, while trotting and galloping were recorded at 250 frames s^{-1}. A minimum of five strides per rat were recorded for each gait–slope combination.

Videos were inspected for quality assurance, including image quality, and presence of all markers in both views. No images were rejected due to quality. Only those that did not provide imaging of the rat MG in both X-ray views over a complete stance phase of a stride cycle were not analyzed. This process reduced our sample to the strides indicated in **Table 1**. The three-dimensional coordinates of each marker for each trial were reconstructed from digitized XY locations of the marker in each pair of frames using XMAlab software v.5.1 (Knörlein et al., 2016). The relevant distances between marker pairs were then extracted following a recently established fluoromicrometry workflow (Konow et al., 2015; Camp et al., 2016; **Figure 2**), the accuracy and precision of which has been validated (see Camp et al., 2016). Time-varying changes in distal fascicle length, muscle length, thickness, width, and free tendon length were calculated and exported to IgorPro (Wavemetrics, Inc., Lake Oswego, OR, United States) for subsequent analysis, together with the timing (based on frame number) of the stance (toe–down) and swing (toe–off) phases of each stride (**Figure 3**).

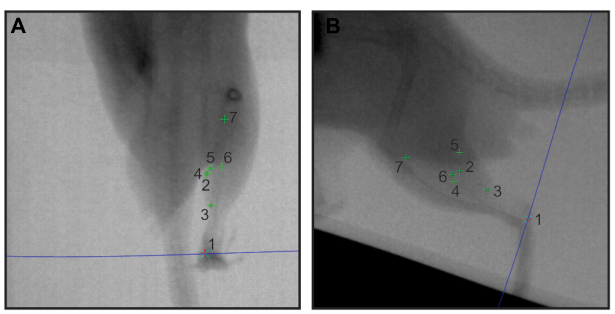

FIGURE 2 | Sample *in vivo* X-ray data. A pair of X-ray stills (**A** – dorsoventral; **B** – lateral) showing digitized radio-opaque markers in the rat MG (numbers 1–7). The blue epi-polar line indicates the direct linear transformation prediction for marker 1, calcaneus. Other markers are: 2, lateral muscle margin; 3, distal myotendinous junction; 4, medial muscle margin; 5, superficial aponeurosis tip; 6, deep aponeurosis tip; and 7, proximal myotendinous junction.

TABLE 1 | Strides analyzed per rat for each gait–slope combination and muscle dimension.

		Slope combination								
Rat	Dimension	Down walk	Down trot	Down gallop	Level walk	Level trot	Level gallop	Up walk	Up trot	Up gallop
11	Length	5	7	5	5	5	6	1	5	4
11	Width	5	7	5	5	5	6	4	–	4
11	Thickness	5	7	5	5	5	6	4	–	4
12	Length	2	7	4	6	–	4	5	7	5
12	Width	5	7	4	6	–	4	5	7	5
12	Thickness	5	7	4	6	–	4	5	7	5
16	Length	–	5	4	5	–	2	6	–	5
16	Width	2	5	4	5	–	2	6	–	4
16	Thickness	2	5	4	5	–	2	6	–	5
18	Length	–	–	2	–	–	6	–	–	2
20	Length	2	–	–	6	3	–	3	3	–
20	Width	2	–	–	7	3	–	3	3	–
20	Thickness	2	–	–	7	3	–	3	3	–
21	Length	7	4	3	6	1	4	4	3	3
21	Width	6	4	1	7	1	4	4	3	3
21	Thickness	6	4	1	7	1	4	4	3	3

Lengthening and shortening strains of the MG fascicles were measured for the stance phase of each stride, where the muscle is activated (Eng et al., 2019). Fascicle strains were normalized by dividing length changes by the average fascicle length measured for each animal during level walking over a complete stride (stance and swing). We report shortening strains as negative and lengthening strains as positive values (**Figure 4**). Shortening and lengthening strains measured between successive image–frames were then summed over each stance phase to quantify net fascicle shortening and lengthening. Active lengthening of fascicles was based on the timing of muscle activation (EMG) and onset of force development (**Figure 3**). Similar measurements of net changes in whole muscle width, thickness and length, and tendon strain were obtained by summing length changes measured between successive image–frames over the stance phase of each stride across all gait–slope combinations.

Muscle–Tendon Force and EMG Measurements

In a separate set of experiments, *in vivo* electromyography (EMG) and MG–tendon force measurements were obtained from three adult male Sprague-Dawley rats of similar weight (275–315 g) trained to move over the nine gait and slope conditions. MG muscle–tendon recordings were made using a custom-fabricated "leaf–spring" tendon force transducer (∼1.7 mm wide × 6.5 mm long; **Figure 1** inset), following the design used to record

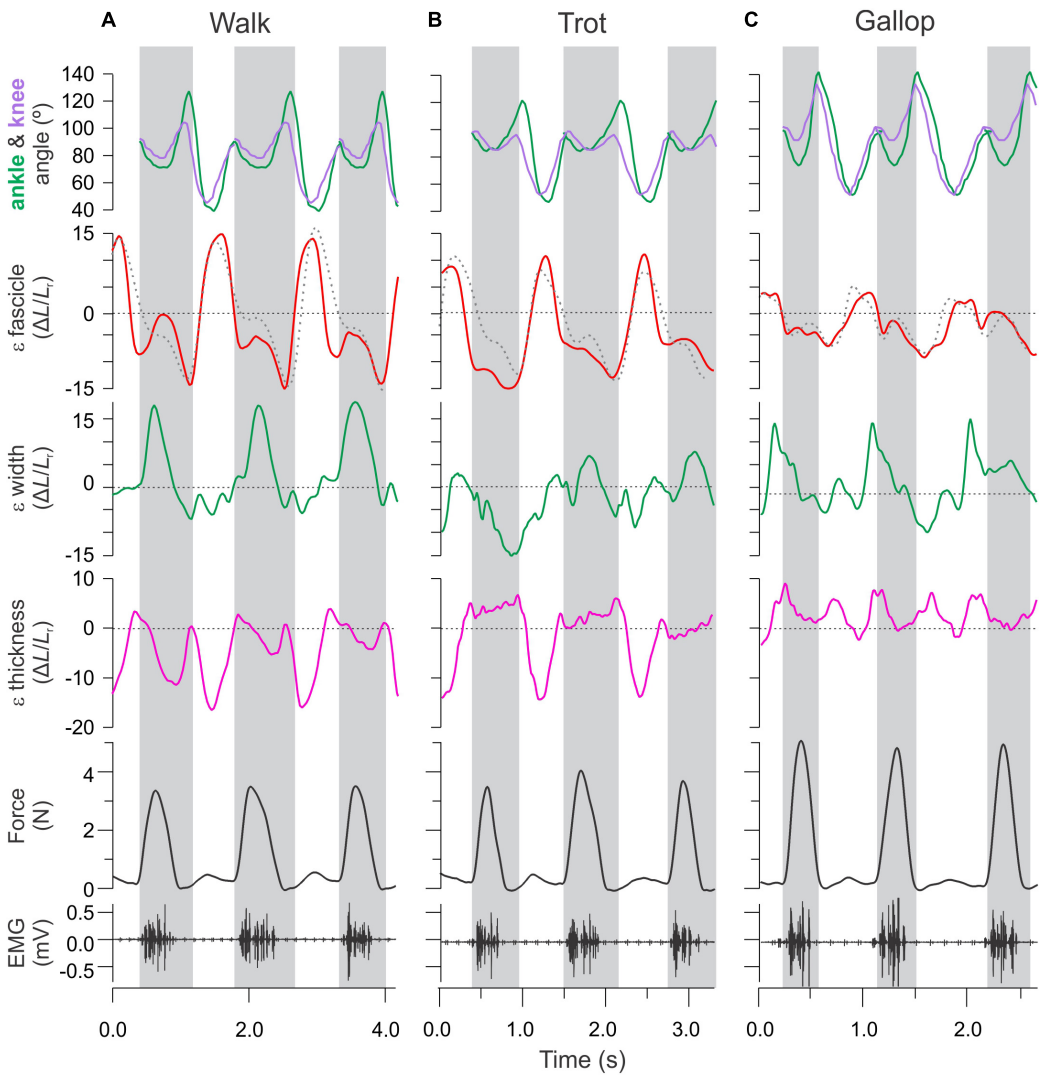

FIGURE 3 | Time varying strain, force, and activation patterns for the rat MG in relation to ankle and knee joint angle changes. Data from **(A)** level walking, **(B)** level trotting, and **(C)** level gallop. Force, activation, and sonomicrometry data are from a different animal than the joint kinematics and strain data. Strain data measured in this study using fluoromicromery (shown as solid traces) are comparable to fascicle length measurements using sonomicromery, the gold-standard approach (shown as dashed traces).

muscle–tendon forces from plantaris longus in *Xenopus laevis* (Richards and Biewener, 2007). Briefly, the leaf–spring tendon transducers were constructed from two thin aluminum strips obtained from the wall of a beverage can, glued together along their length using cyanoacrylate adhesive (Loctite Corp., Avon, OH, United States), with a small metal foil strain gauge (FLK-1-11, Tokyo Sokki Kenkyujo, Ltd., Tokyo, Japan) bonded using cyanoacrylate adhesive to the concave surface of the curved leaf–spring (**Figure 1**). Following this, 36-gauge lead wires were soldered to the strain gauge and insulated with epoxy. Two short lengths (∼6 cm) of 4-0 silk suture (Ethicon, Inc., Somerville, NJ, United States) were also epoxied to the surface of either end of the transducer (to anchor the convex surface of the transducer against the tendon when implanted). The entirety of the transducer was then coated with M-Coat A (polyurethane curing agent, Micromeasurements, Inc., Raleigh, NC, United States) to further seal and insulate the circuit, eliminate adverse tissue reaction, and minimize tendon chafing. The shallow curvature of the aluminum functions as a leaf–spring to allow tensile muscle force transmitted via the tendon of the muscle to be measured by the strain gauge as the leaf–spring is deflected under the applied load.

Bi-polar offset hook EMG electrodes (1 mm bared tip; 1.5 mm spacing) were constructed from insulated 0.1 mm silver wire (California Fine Wire, Grover Beach, CA, United States), implanted mid-belly, and sutured to the muscle's epimyseal surface with 5-0 silk. The EMG electrodes and leaf–spring tendon force transducers/lead wires were disinfected in Cetylcide^{TM} solution (Cetylite, Inc., Pennsauken, NJ, United States) and rinsed repeatedly in sterile water before implantation. Lead wires

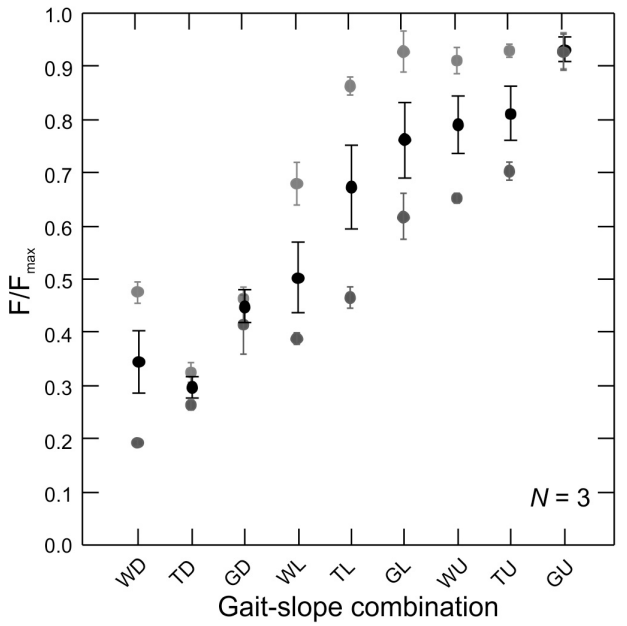

FIGURE 4 | Force production by the rat MG across gait–slope combinations. Gray tones are subjects ($N = 3$). Circles represents the mean of all recorded strides for a given subject (**Table 1**). Error bars are mean ± SD. There is a statistically significant relationship between muscle force production and gait–slope combination (LMM: $t_{2,336} = 22.58$; $p < 0.0001$).

were provided slack and passed subcutaneously to an epoxy insulated, custom-designed micro-connector (GM-6, Microtech, Inc., Boothwyn, PA, United States) that was anchored to the skin overlying the neck using 3-0 Vicryl suture (Ethicon, Inc., Somerville, NJ, United States).

Muscle shape changes, fascicle strains, and tendon strains were related to changes in force requirements across gait and slope combinations based on *in vivo* recordings of MG–tendon force (**Figures 1**, **4**).

Implant Verifications and Measurement Calibrations

Following the *in vivo* experiments, subjects were deeply anesthetized (Isoflurane 4% in induction chamber), and euthanized (intra-cardiac overdose injection of sodium pentobarbital). For subjects used for bi-planar high-speed X-ray experiments, the implanted hind limb was dissected free, carefully de-gloved, fixed in 10% phosphate-buffered formalin, and soft-tissues of the whole limb were contrast-stained in a 4% alcoholic solution of Phosphomolybdenum acid, and micro-CT scanned (Bruker 1670). Volume segmentation of scans enabled us to measure the error in distance measurements caused by markers not being lodged precisely at the muscle borders (for muscle width and thickness) or myotendinous junctions (fascicle and whole muscle length). Where needed, segment corrections using the residual distances obtained from the scans were performed on the raw distance data.

For subjects used to obtain muscle–tendon force recordings, a 4-0 silk suture was tied to the calcar, which was then severed from the foot to release the distal MG attachment. The suture was attached to a load-cell (Kistler 9203) and a series of pull calibrations were obtained, ensuring that the voltage levels recorded *in vivo* were reached. By regressing the time-varying load-cell (N) and strain gauge (V) signals against each other, we obtained the slope of the linear relationship with $R^2 > 0.96$ (**Supplementary Figure S2**), which was used to calibrate our *in vivo* tendon strain gauge measurements to force (N) (Gabaldon et al., 2004; Richards and Biewener, 2007). The quality of fit to a linear relationship between force and voltage output of the transducers used here matches that obtained from the anuran plantaris tendon (Richards and Biewener, 2007), as well as "E"-shaped buckle transducers used to measure muscle tendon forces in cats (Walmsley et al., 1978), guinea fowl (Daley and Biewener, 2003), goats (Walmsley et al., 1978; McGuigan et al., 2009), wallabies (Biewener and Baudinette, 1995), and strain-force recordings of calcified turkey tendons (Gabaldon et al., 2004).

Statistics

We used linear mixed models (Systat Software, Inc., San Jose, CA, United States), factoring gait–slope combination (a categorical variable with nine states) and individual as random effects, and intercepts of the model as fixed effect, to determine the effect of gait and slope on our stride–specific, dependent variables; MG peak stance force; fascicle strain, tendon strain, and muscle shape (width and thickness changes) (**Table 2**). Means ± SD were also calculated for each variable for all gait and slope combinations.

RESULTS

Measurements of *in vivo* MG–tendon forces (normalized to the peak force recorded for each animal, F_{max}) across the nine gait and slope combinations showed a statistically significant pattern (LMM: $t_{2,336} = 22.58$; $p < 0.0001$) of force increase from downhill walking to uphill galloping (**Figure 4**). Moreover, MG–tendon force increased with gait changes from walking, via trot, to gallop ($t_{1,452} = 72.28$; $p < 0.0001$) and with changes in slope from downhill, via level, to uphill ($t_{1,336} = 20.21$; $p < 0.0001$).

MG fascicle strains measured via fluoromicrometry across slope and gait conditions exhibited generally consistent patterns when compared across slopes with respect to gaits (**Figure 5A**) but more complex patterns when compared across gaits with respect to slope (**Figure 5B**). MG fascicles underwent active lengthening strains during early stance where the center-of-mass is being decelerated and the ankle joint flexes and shortening strains during the second half of stance where the center-of-mass is being re-accelerated and the ankle joint re-extends (**Figure 3**). As a result, net fascicle strains were generally less than either lengthening or shortening strains for all gait–slope combinations, except for level walk (**Figure 5**). Net MG fascicle strains ranged from 2.0 ± 3.5% (mean ± SD) to 0.8 ± 5.3% for all downhill gaits, from 3.5 ± 11.2% to -4.5 ± 1.9% during level gaits, and from 0.1 ± 7.4% to -8.0 ± 4.2% for uphill gaits. Level and uphill walking gaits exhibited the greatest variability in MG

TABLE 2 | Summary linear mixed model statistics for relationships between gait–slope combinations and the fixed and random effects factored.

	Fixed effect (intercept)			Random effect (G–S combo)			Random effect (individual)		
	Estimate (lower–upper 95% CI)	SE	*P*	Estimate (lower–upper 95% CI)	SE	*P*	Estimate (lower–upper 95% CI)	SE	*P*
F_{MG}	0.341 (0.232–0.45)	0.056	***	0.089 (0.081–0.096)	0.004	***	−0.006 (−0.009 to −0.004)	0.001	***
V_{muscle}	−0.670 (−1.133 to −0.207)	0.235	**	−0.089 (−0.161 to −0.018)	0.036	**	0.001 (−0.008 to 0.009)	0.004	n.s.
$\varepsilon_{thickness}$	−0.689 (−0.731 to −0.647)	0.021	***	−0.013 (−0.017 to −0.009)	0.002	***	−0.030 (−0.037 to −0.023)	0.003	***
ε_{width}	−0.598 (−0.671 to −0.524)	0.037	***	0.017 (0.010–0.024)	0.003	***	0.014 (0.009–0.018)	0.002	***
ε_{tendon}	0.094 (0.050–0.138)	0.014	**	0.006 (0.001–0.008)	0.002	***	−0.033 (−0.004 to −0.001)	0.001	**

*Please see section "Results" for t-statistics. ***P = 0.001; **P = 0.01.*

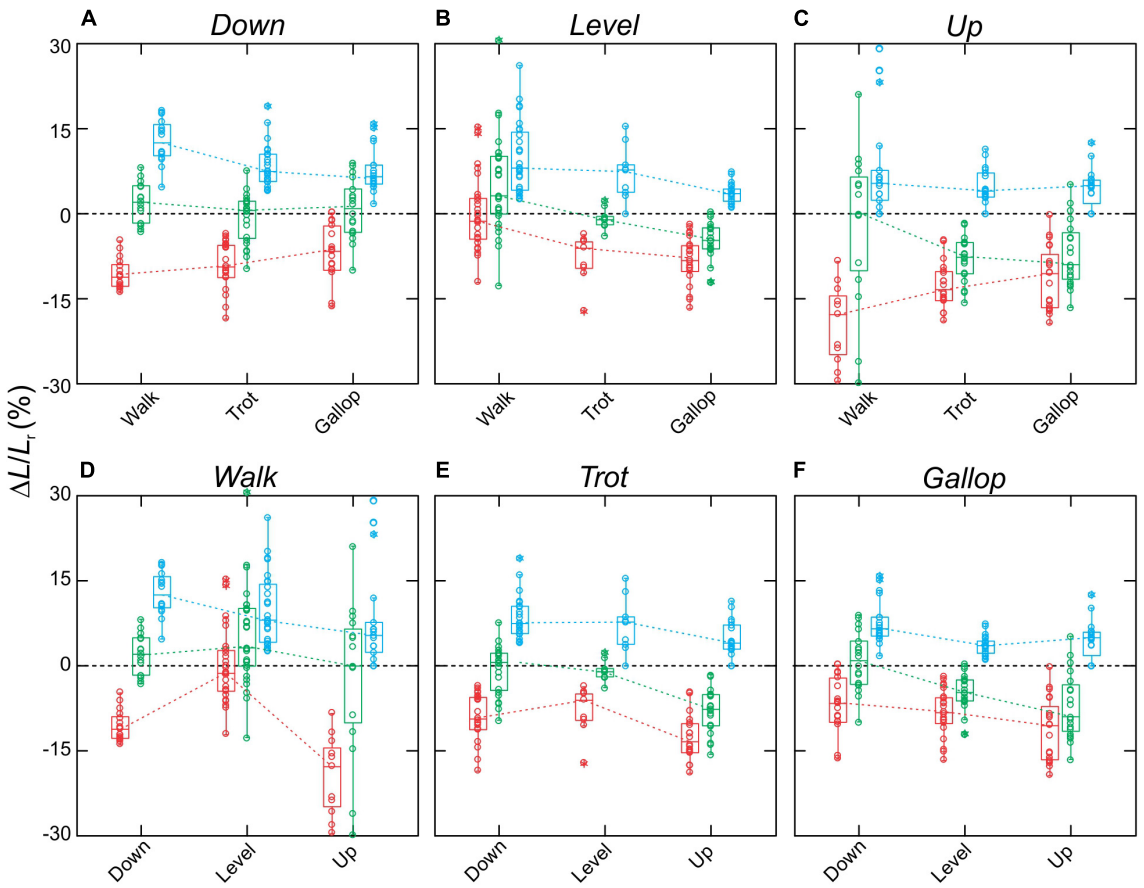

FIGURE 5 | Fascicle strains for the rat MG. Data shown **(A–C)** across slopes, with respect to gaits, and **(D–F)** across gaits, with respect to slopes. Boxes show median with 75th and 25th quartiles, whiskers are the data range for all strides of all subjects pooled. Dot-density circles are stride-specific data points. Red indicates shortening strains, blue are lengthening strains, and green are net strains.

fascicle strains, consistent with the less steady locomotor behavior exhibited by the rats during walking. Except for walking, both trotting and galloping gaits exhibited increased net MG fascicle shortening as animals transitioned from downhill to level to uphill gait (**Figure 5B**).

When compared across all gait–slope combinations, the pooled data exhibited a significant decrease in changes of rat MG thickness ($t_{1,140} = -6.90$; $p < 0.001$), increase in changes in MG width ($t_{1,154} = 5.08$; $p < 0.001$), and a reduction in MG muscle belly contraction velocity ($t_{1,336} = 22.58$; $p < 0.001$) with increased force, as driven by changes in gait and slope conditions that alter the force generating requirements of the muscle (**Figure 6**).

Fluoromicrometry measurements of MG tendon strain exhibited a significant increase ($t_{2,67} = 2.53$; $p < 0.014$) with respect to gait–slope combination (**Figure 7**) that paralleled the increase in MG–tendon force (**Figure 3**). Such an increase is expected, given the passive elastic properties of tendon. MG tendon strains increased from 1.95 ± 0.50% (mean ± SD) during downhill walking to 5.82 ± 1.12% during uphill galloping.

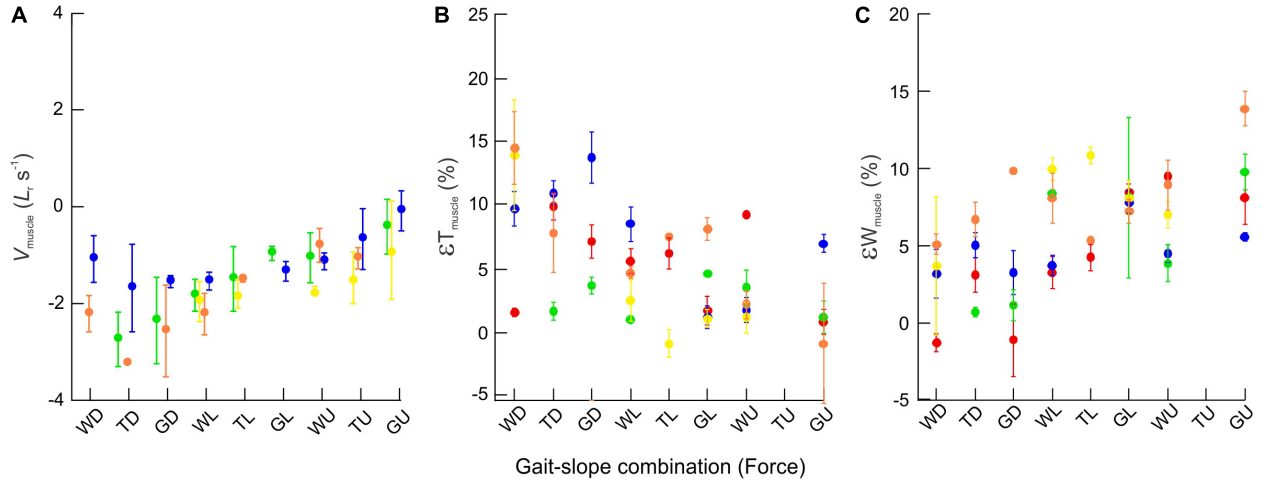

FIGURE 6 | Bulging dynamics of the rat MG. Scatter plots showing changes across gait–slope combinations in **(A)** muscle contraction velocity (normalized to resting muscle fascicle lengths per second) **(B)** εT_{muscle}, and **(C)** εW_{muscle}. Colors represent subjects and each dot indicates the mean of all strides for a particular gait–slope combination, with error bars giving one standard error around the mean. There are statistically significant relationships between all three response variables with respect to gait–slope combinations (LMM: V_{muscle}; $t_{1,336} = 22.58$; $p < 0.001$. εT; $t_{1,140} = -6.90$; $p < 0.001$. εW; $t_{1,154} = 5.08$; $p < 0.001$).

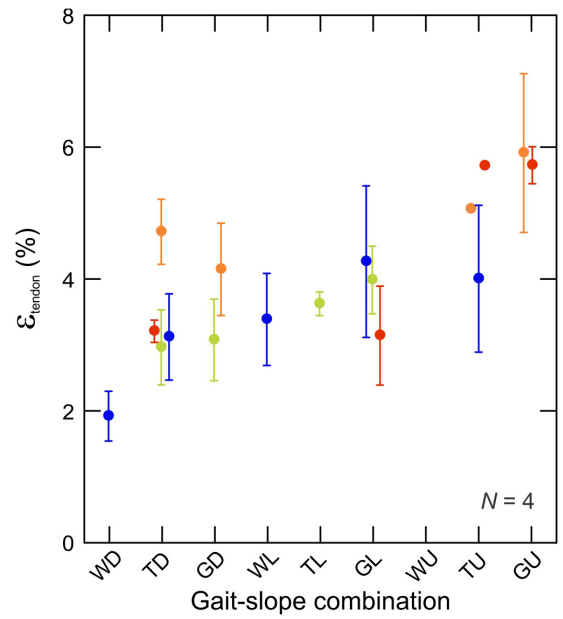

FIGURE 7 | Tendon strain during the force-rise period of stance. Data are mean ± SEM and colors indicate subjects ($N = 4$). There is a statistically significant relationship between tendon strain and force production (by proxy of gait–slope combination) ($t_{2,336} = 22,58$; $p < 0.0001$).

DISCUSSION

Our fluoromicrometry results for the MG muscle–tendon unit of healthy young adult rats, obtained across a range of gait and slope conditions that require changes in MG–tendon force, support our hypothesis that the rat MG preferentially bulges in width during high-force contractions (uphill gallop) to augment whole muscle force, but preferentially bulges in thickness to increase whole muscle shortening velocity during low-force contractions (downhill walk). Similar shifts in muscle belly shape change were observed within the human MG across a range of low torque to high torque pedaling conditions (Dick and Wakeling, 2017) and are consistent with earlier *in situ* force–velocity measurements of the turkey LG showing increased fascicle rotation to facilitate whole muscle shortening during low-force contractions, but restricted fascicle rotation during high-force contractions (Azizi et al., 2008). In the case of pedaling, shape changes of the human MG were force-dependent but not velocity-dependent across different torque and cadence conditions (Dick and Wakeling, 2017). For the rat MG, we observe force-dependent, as well as whole muscle velocity-dependent, dynamic changes in muscle shape.

Fascicle MG strains recorded via fluoromicrometry were generally similar to the patterns of strain recorded in separate experiments using sonomicrometry across gait and level versus incline conditions (**Figure 3**; Eng et al., 2019), offering further validation of fluoromicrometry as a viable method for assessing fascicle strain patterns in relation to muscle shape changes (Camp et al., 2016). Although changes in muscle belly thickness and width were correlated with changes in muscle force linked to the varying gait–slope combinations, fascicle strain patterns varied more consistently with changes in slope than with gait. Fascicle lengthening strains during the first half of stance (force development) and shortening strains during the second half of stance (force relaxation) generally exceeded net fascicle strains across nearly all gait–slope conditions (except for level walk). Overall, net fascicle strains (measured distally within the muscle) were small (±1.2%) across gaits during downhill locomotion, varied from net lengthening strain during level walking (3.5 ± 11.2%) to net shortening strain during level

galloping −4.5 ± 1.9%, and exhibited the largest net shortening strains during uphill trotting (-8.0 ± 0.4%) and galloping (−9.0 ± 0.6%). The limited net MG fascicle strains during level gait and increase in net shortening strain during incline trot and gallop generally match the patterns observed based on sonomicrometry (Eng et al., 2019). These patterns indicate limited net fascicle strain and work output of the rat MG during level locomotion, but increased shortening and work during uphill gait.

Tendon strains showed a statistically significant relationship with force development as strains increased with the increase in force requirements associated with the different gait–slope conditions. This finding indicates an increase in the role of elastic energy return from downhill walk to uphill gallop and adds to the debate about the role of tendon compliance in small mammal locomotion (Ker et al., 1988; Bullimore and Burn, 2005). The strains we recorded for the rat Achilles tendon *in vivo* match well with the data from recent materials testing of the same tendon (Javidi et al., 2019). Because MG fascicle strains showed little evidence of net lengthening across all gait–slope conditions, energy stored in the MG tendon during force development is presumably recovered to power limb and body movement, rather than being dissipated by doing work on the muscle (via fascicle stretch; i.e., "elastic backfire"; *sensu* (Roberts and Azizi, 2011); in contrast to the buffering of rapid stretch and energy absorption observed for turkey landings from drops of different heights (Konow and Roberts, 2015) and for landings from human jumps (Werkhausen et al., 2017; Werkhausen et al., 2018; Helm et al., 2019; Hollville et al., 2019).

Our fluoromicrometry results for young adult rat MG shape change are also consistent with those reported for healthy young versus old rats based on *in situ* sonomicrometry and ergometry measurements of fascicle and whole muscle length change and muscle force (Holt et al., 2016). Whereas the MG of healthy young rats exhibited variable gearing, increased MG connective tissue stiffness in aged rats resulted in no change in muscle gearing (ratio of whole muscle velocity: fascicle velocity). Although measurements of muscle thickness and width were not obtained directly by Holt et al. (2016), the increased gearing for low-force isotonic contractions was interpreted as being facilitated by an increase in muscle thickness (resulting from fascicle rotation), as we observe here. Under high-force isotonic contractions, the reduction in gearing was interpreted as a preferential change in muscle width to preserve force output of the muscle by restricting a change in muscle thickness (Holt et al., 2016). Again, our results for muscle shape change during unrestrained locomotion across gait–slope combinations requiring changes in muscle force requirements support the *in situ* pattern observed for the MG of young adult rats, in contrast to the pattern observed for aged rats.

Prior ultrasound work on humans has characterized muscle architectural dynamics, initially in 2D (e.g., Aggeloussis et al., 2010; Giannakou et al., 2011) and recently in 3D, using bi-planar ultrasound (Raiteri et al., 2016) or by shifting one ultrasound probe to measure muscle thickness and width alternatively in carefully controlled dynamometer studies (Randhawa and Wakeling, 2018). Measurements of MG thickness bulging are relatively common (Dick and Wakeling, 2017; Hodson-Tole and Lai, 2019), and reported thickness strains of approximately 14% for the human MG are in-line with our measurements from the rat MG. By contrast, transverse (widthwise) muscle bulging is less commonly measured *in vivo* (Raiteri et al., 2016; Randhawa and Wakeling, 2018), and available data appear to vary with muscle architecture and function. Widthwise strains of approximately 28% for the human MG (Randhawa and Wakeling, 2018) are in line with our measurements from the rat MG. However, in a recent study of the human tibialis anterior (a bi-pennate ankle dorsiflexor), there was no relationship between contraction intensity and muscle widthwise bulging, which overall was limited (Raiteri et al., 2016). It is also noteworthy that compared to the rat and human MG, the human TA exhibits very different shape–change dynamics in relation to muscle and fascicle length–change as contraction intensity increases (Raiteri et al., 2016).

Potential Limitations of the Study

Our study of how *in vivo* shape changes of the rat MG interact with variation in muscle force to adjust muscle work and power output across gait and grade conditions depends on the simplifying assumption that the transverse plane across which width and thickness length changes were measured remained uniform with respect to overall changes in muscle length. Additional markers would need to be implanted to evaluate whether this assumption remains valid during *in vivo* skeletal muscle contractions. Whether or not this is the case may also vary across muscles having differing architecture. Surgical interference of overlying fascia to implant the small tantalum markers may also have compromised the integrity of the intact muscle in its native state, influencing the dynamics of the muscle's bulging during contraction. However, fluoromicrometry measurements of muscle length (and shape) change based on small trochar-implanted tantalum spheres has considerable advantage compared with more invasive dissections required to access muscles and implant sonomicrometry crystals—another method commonly used to obtain direct *in vivo* recordings of muscle length change—which require penetrating the epimyseal lining of a muscle and anchoring lead wires to the muscle's surface. Ultrasound-based methods (e.g., Aggeloussis et al., 2010; Giannakou et al., 2011; Raiteri et al., 2016; Dick and Wakeling, 2017), while clearly the best and most suitable method for *in vivo* assessments of human skeletal muscle length and shape change, depend on reliable anchoring of the ultrasound probes to minimize skin movement artifacts relative to the underlying muscle and adequate image quality obtained from superficial muscles. Further, ultrasound methods are not feasible for smaller animal studies, which also allow direct measurements of muscle–tendon force.

Finally, our use of a leaf–spring tendon force transducer depends on a linear and stable calibration of force with respect to voltage output from the transducer's strain gauge. By calibrating the transducers immediately after obtaining *in vivo* muscle–tendon force recordings, we minimize the risk that our transducer calibration is not an accurate recording of *in vivo* force. The calibrations of this custom-designed transducer (**Supplementary Figure S2**) match well the linear fits obtained

using "E"-shaped buckle transducers (Walmsley et al., 1978; Biewener and Baudinette, 1995; Daley and Biewener, 2003) and strain gauges bonded directly to calcified tendons (Gabaldon et al., 2004), and their design allows for recording *in vivo* forces in very small tendons, which are not amendable to bulkier "E"-shaped buckle transducers.

CONCLUSION

Our measurements of muscle shape change dynamics during unrestrained locomotion across differing gait–slope combinations based on 3D X-ray imaging and fluoromicrometry support the findings of past studies (Azizi et al., 2008; Holt et al., 2016) that have inferred muscle shape change based on *in situ* force–velocity measurements and how changes in force output affect fascicle rotation and the gearing of pennate muscles. Thus, increased gearing by bulging of muscle thickness, allowing fibers to rotate under low-force conditions, versus reduced gearing by preferential bulging of muscle width to favor higher force output generally appears to apply to both controlled *in situ* muscle contractile experiments as well as the dynamics of muscle shape change that occur during human pedaling (Dick and Wakeling, 2017), similar to the unrestrained locomotion across gait and slope conditions studied here. Our data on how interactions among muscle shape, fiber length change, muscle force, and connective tissue behavior affect the dynamics of muscle work across varying motor tasks expand our understanding of how the dynamics of these interactions broaden the functional repertoire of whole muscles in relation to their underlying force–velocity and force–length properties. This understanding will benefit musculoskeletal modeling approaches by increasing their abilities to account for how dynamic changes in muscle shape influence whole muscle shortening and work.

DATA AVAILABILITY STATEMENT

Data used in this study are available from the authors upon request.

ETHICS STATEMENT

The animal study was reviewed and approved by Harvard University's FAS Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

NK and AB contributed to the study design. NK contributed to the data acquisition. AC contributed to the tracking. NK and AC contributed to the data organizing, analyses, and statistical testing. NK, AC, and AB contributed to the manuscript draft, editing, and approval.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00143/full#supplementary-material

FIGURE S1 | Full-frame version of the image pair used for **Figure 2**. Digitalization markers were removed so that radio-opaque markers can be seen. Each image is 1024 × 1024 pixels.

FIGURE S2 | (A) Time-varying signals obtained during pull-calibration of strain-gauge (ε[V]) against the Kistler load-cell (N) for one of the rats. **(B)** Three examples of regression lines (thick, black) fitted to the data for force-rise (see panel **A**), showing the equation and R^2 ($\geq$0.96) for each fit.

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Loading Rate Has Little Influence on Tendon Fascicle Mechanics

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Mechanically, tendons behave like springs and store energy by stretching in proportion to applied stress. This relationship is potentially modified by the rate at which stress is applied, a phenomenon known as viscosity. Viscoelasticity, the combined effects of elasticity and viscosity, can affect maximum strain, the amount of stored energy, and the proportion of energy recovered (resilience). Previous studies of tendons have investigated the functional effects of viscoelasticity, but not at the intermediate durations of loading that are known to occur in fast locomotor events. In this study, we isolated tendon fascicles from rat tails and performed force-controlled tensile tests at rates between ∼10 MPa s^{-1} to ∼80 MPa s^{-1}. At high rates of applied stress, we found that tendon fascicles strained less, stored less energy, and were more resilient than at low rates of stress (p = 0.007, p = 0.040, and p = 0.004, respectively). The measured changes, however, were very small across the range of strain rates studied. For example, the average strain for the slowest loading rate was 0.637% while it was 0.614% for the fastest loading. We conclude that although there is a measurable effect of loading rate on tendon mechanics, the effect is small and can be largely ignored in the context of muscle-actuated locomotion, with the possible exception of extreme muscle-tendon morphologies.

Keywords: viscoelasticity, tendon fascicle, rat tail, elastic energy (EE), resilience

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INTRODUCTION

Nearly all biological structures, including those that comprise the musculoskeletal system, are viscoelastic – capable of deforming in response to load and reforming in its absence (elasticity) while exhibiting some sensitivity to load-rate (viscosity). For example, applying tensile strain at a constant rate to ligaments of rabbits (Crisco et al., 2002), monkeys (Noyes et al., 1974), and humans (Pioletti et al., 1998; Funk et al., 2000; Bonifasi-Lista et al., 2005) generally results in typical stress-strain curves: a relatively low stress "toe region" followed by linear increase in stress in response to strain (**Figure 1A**). These relationships vary, however, with strain rate. Ligaments, in general, exhibit higher slopes of stress-strain when faster strain rates are applied (**Figure 1A**; Haut and Little, 1969; Noyes et al., 1974; Crisco et al., 2002) and, when held at constant length after stretching, increasingly relax and dissipate stored energy the longer they are held (**Figure 1B**; Bonifasi-Lista et al., 2005). In addition to ligaments, rate-dependence has important mechanical consequences in musculoskeletal structures such as bones (Black and Korostoff, 1973; Lakes et al., 1979; Rimnac et al., 1993; Bowman et al., 1994). Because the ratio of viscous to elastic behavior varies with rate, the rate of loading has the potential to affect mechanical output.

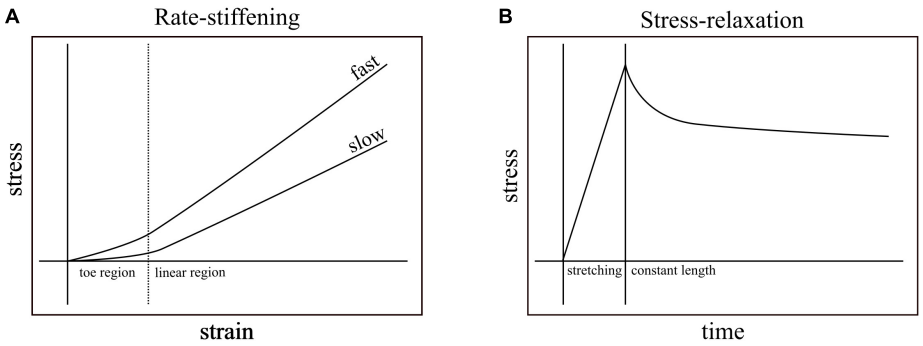

FIGURE 1 | Rate-stiffening and stress-relaxation are two mechanical behaviors that result from the viscoelasticity of biological structures. **(A)** In rate-stiffening, faster rates of strain increase the effective stiffness of the structure, resulting in higher stress for a given strain. These effects can be seen in both the toe region (left of dotted line) and the linear region (right of dotted line). **(B)** Stress-relaxation results from holding a structure at constant length (right of solid line) upon the completion of stretching. Over time, the stress required to maintain constant length is reduced as the energy stored in the structure dissipates into the environment.

Given that musculoskeletal structures are viscoelastic, tendons that are involved in multiple locomotor functions may modulate their mechanics in response to loading rate. In other words, if tendons are sensitive to loading rate, despite being completely passive structures, they may support different functions depending on the circumstances in which they are loaded. Hypothetically, this may manifest in a single tendon that can paradoxically enhance both energy storage and energy dissipation depending on whether force is provided by a slow contraction from the muscle or a fast impact from the ground.

Studies of the mechanical behavior of tendon have established viscoelastic behavior. The most common measurement of viscoelasticity involves stress-relaxation studies, which show declines in force in tendons that are stretched and then held at constant lengths (**Figure 1B**). These studies focus on the dissipative nature of tendons after loading (Tanaka et al., 1999; Provenzano et al., 2002; Elliott et al., 2003; Bonifasi-Lista et al., 2005; Duenwald et al., 2009), and rarely vary loading rate within a single study. While these studies provide insight into the viscoelastic effects that dominate at relatively long time scales (e.g., minutes), they do not typically test the viscoelastic effects that dominate during transient loading events such as the high impact landings that load turkey tendons within ∼60 ms (Konow et al., 2012) and the stretching of tendons prior to power amplification within ∼250 ms (Wainwright and Bennett, 1992; Lappin et al., 2006; Van Wassenbergh et al., 2008). Additionally, while some studies measured the effects of viscoelasticity while varying loading rate in collagenous structures, the two studies that are closest to matching rates found in transient events are still either slower (Robinson et al., 2004; load to failure in ∼400 ms) or faster (Crisco et al., 2002; load to failure in ∼4.6 ms) than physiological durations of loading that do not cause injury.

We tested viscoelasticity in the relatively unspecialized fascicles of rat tail tendons. Whole tendons can exhibit complex geometry along their lengths, thereby making stress calculations problematic; but, tendon fascicle geometry is relatively simple and more amenable to material testing. Additionally, there is a large body of work on rat tail tendon fascicles to which we can compare our results (Rigby et al., 1959; Screen et al., 2003; Haraldsson et al., 2009; Bruneau et al., 2010; Legerlotz et al., 2010). By isolating tendon fascicles and replacing their connected muscles with a dual force muscle motor, we were able to test how muscle-tendon interactions are affected by variation in loading rates that occur during transient events. Although viscoelasticity is traditionally measured by controlling strain, variation in tendon strain in a muscle-tendon unit is controlled by the muscle, a contractile element that acts as a force generator. Therefore, we programmed the dual mode motor to simulate muscle action by varying force directly, enabling fine control of both the amount and rate of force applied to the tendon fascicle at any given moment. We tested whether applying tensile stress at varying rates resulted in significant changes in three mechanical behaviors of the tendon fascicles: maximum strain, energy storage, and resilience.

MATERIALS AND METHODS

The tails of seven adult rats, which were euthanized for reasons unrelated to this study, were isolated for dissection and immediately submerged in Mammalian Ringer's solution (115.00 mM NaCl, 4.70 mM KCl, 2.00 mM $CaCl_2$, 1.20 mM $MgSO_4$, 40.00 mM TRIS, 10.00 mM TRIS.HCl). Using a previously published method of dissection and extraction (Bruneau et al., 2010), from each tail, at least 20 tendon fascicles were dissected, wrapped in Ringer's-soaked gauze, and kept frozen until tested.

Prior to testing, tendon fascicles were thawed at room temperature and immediately placed in mammalian Ringer's solution for at least 10 min. Tendon fascicles were trimmed to a total length of ∼50 mm. We modified a previously published method of attaching the tendon fascicle to the testing chamber (Rigby et al., 1959). While submerged in solution, a single tendon fascicle was manipulated at both ends via forceps to form a half-loop. Both free ends of the tendon fascicle were gripped by a flat-faced copper clamp lined with Emery paper (**Figure 2**). Fascicles were transferred to an acrylic testing chamber filled with Ringer's solution. In the chamber, the miniature clamp was

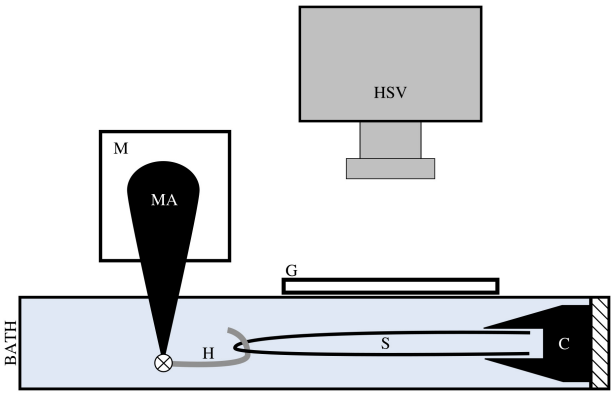

FIGURE 2 | We applied tensile stress to a tendon fascicle sample (s) via a muscle motor (m) while recording video of the sample at 1000 Hz with a high speed video camera (HSV). We formed a loop with the tendon fascicle, gripped the two free ends in a clamp (c), and connected a hook (h) through the fascicle sample loop. The hook, which attached to the motor arm (MA) via a pin joint, was lined with plastic tubing to reduce friction on the sample during testing. During testing, the hook, fascicle, and clamp were fully submerged in mammalian Ringer's solution. To prevent ripples at the surface of the solution, we placed a glass slide (g) directly above the fascicle sample.

secured to the acrylic wall, and a plastic-lined sterling silver hook was passed through the open loop of the fascicle. This hook was directly connected to the arm of a dual mode muscle level (305C-LR; Aurora Scientific; Ontario, Canada) via a pin joint. To ensure the tendon fascicle was not over-stretched during manipulation, we applied a preload of ∼0.050 N to the tendon fascicle and programmed the muscle motor to not exceed this force for the duration of preparation. Directly above the tendon fascicle, we placed a glass slide and added additional Ringer's solution to the chamber until the liquid touched the bottom of the glass slide to prevent surface ripples during mechanical testing.

We calculated stress as the load divided by cross-sectional area at preload; therefore, an accurate estimate of cross-sectional area was required. We estimated the average cross-sectional area using fascicle width measured from a pair of orthogonal images. The testing chamber included a mirror that formed a 45-degree angle with respect to the horizontal surface of the testing chamber. We included two independent reference objects in the chamber for length calibration, one for each view. High speed video was captured from above via camera (Fastcam-X 1280PCI; Photron, IN, United States) mounted to a microscope (GZ6E; Leica, IL, United States).

Fascicle cross-sectional area was modeled as an ellipse using the following equation:

$$A = \pi \cdot a \cdot b \quad (1)$$

where a and b are equal to half the width of the fascicle in each of the orthogonal views. To estimate the width of the fascicle, each image was manually preprocessed and were imported into R (R 3.3.1, Vienna, Austria) where estimates of a and b were calculated.

Previous studies have shown that strain calculated using motor distance can overestimate within-tendon strain by as much as 6% due to slipping at clamped surfaces (Haraldsson et al., 2005). Therefore, instead of relying on motor displacement for strain calculations, we measured strain using a non-contact, optical method. In preparation for visual strain measurement, we marked the tendon fascicle with multiple ink dots spaced roughly 5 mm apart. Because the tendon fascicle was looped, it formed two separate "strands" on which we placed dots. We offset the dots on each strand to provide an immediate visual indicator of slipping and failure during tests.

In all trials, we input different levels of stress while measuring the resulting strain. Tendon fascicles were preloaded to ∼0.050 N, which did not result in any visible changes in the crimping of the fascicles. We then inputted a triangle wave to control force such that a maximum of 4 MPa of stress was applied to the tendon fascicle. We chose a maximum stress of 4 MPa because previous studies have reported that under this stress, tendon fascicles remained linear, and plastic deformation was unlikely when tested repeatedly (Haraldsson et al., 2005). The duration of the rising portion of the triangle wave was experimentally varied from 50 to 400 ms at intervals of 50 ms to capture the natural range of variation in tendon loading. The duration of the falling portion of the triangle wave was fixed at 100 ms for all trials. Preliminary results showed no evidence of history effects; therefore, before each replicate, the order of these durations was randomized. Between each sequence of loading-unloading, the tendon fascicle rested in solution for at least 5 min, which provided enough time for the fascicle to recover from stress-relaxation effects. We collected data until either the tendon fascicle broke (replicates in which plastic deformation was obvious were removed prior to analysis) or at least three replicates of tensile stretch were collected. During each trial, we recorded high speed video at 1000 fps, which was synchronized with the muscle motor. In order to capture the resting length of the tendon fascicle, the high speed camera began recording 100 frames (100 ms) prior to each test (**Figure 3**).

Using a custom point tracker developed in R, we converted the synchronized high speed camera into a visual tensometer. The marks that were farthest apart on a single fascicle strand were selected manually by identifying a single pixel in each point. Because the boundaries formed by the ink mark were sensitive to optic parameters (lighting, lens effects, discretization of pixels etc.), we tracked the centroid of each marker, which was calculated as the mean x and y location of each pixel in each marker. This had two advantages. First, it minimized errors that resulted from inconsistent boundary identification. Second, because ink marks spanned multiple pixels in each image, calculating the centroid of a body of pixels allowed sub-pixel resolution of marker position.

After the centroids of both points were tracked, we calculated the distance, in pixels, between the centroids of each frame using the following equation:

$$distance = \sqrt{(c_1^x - c_2^x)^2 + (c_1^y - c_2^y)^2} \quad (2)$$

where c_1^x and c_2^x are the x values of the centroid of points 1 and 2, respectively, and c_1^y and c_2^y are the y values of the centroid of points 1 and 2, respectively. Finally, the distance between each

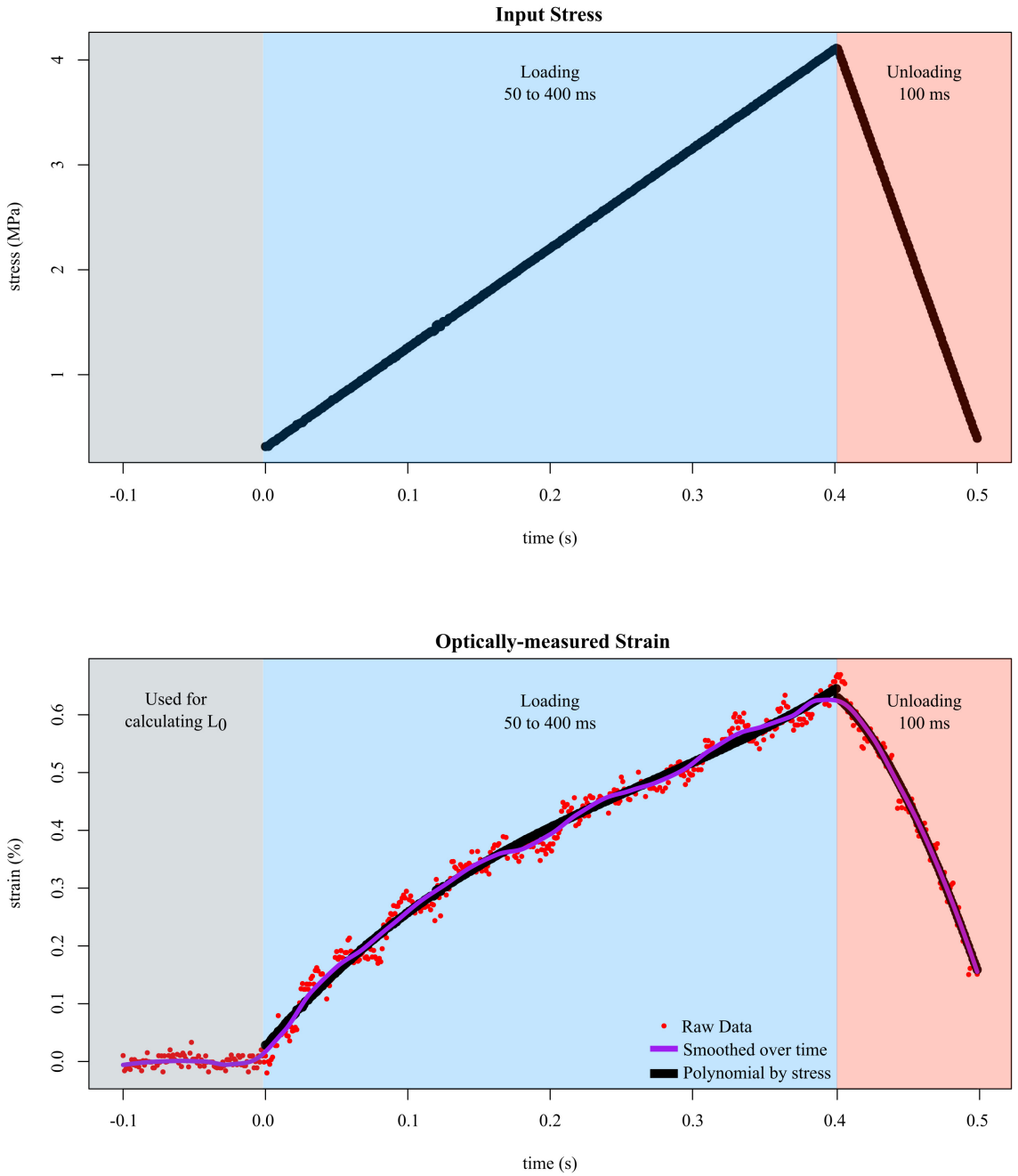

FIGURE 3 | Tendon fascicles were linearly loaded to ∼4 MPa of stress at different rates while strain was optically-measured. To determine the mechanical response to stress rate, the duration of loading **(blue background)** was experimentally varied between 50 and 400 ms. After loading, the tendon fascicle was unloaded to its original stress within 100 ms in preparation for the next trial **(red background)**. Calculating strain required dividing length by L_0, which was defined as the average distance between the markers over 100 ms prior to input stress **(gray background)**. The raw strain data **(red dots)**, were then smoothed via LOESS over time. The final phase of smoothing involved fitting 3rd order polynomials **(black lines)** to the stress-strain data of the loading data.

marker was converted to strain by dividing each distance by the average distance between the markers recorded 100 ms prior to the start of each test.

The data were filtered in two ways. First, with respect to time, the position of each marker used to calculate strain was smoothed using Local Polynomial Regression Fitting (loess function in R) using an arbitrary smoothing parameter of 0.2. These data were then combined with stress, which was measured from the muscle motor. The dataset was then subdivided into the loading and unloading phases. Second, to each subset, 3rd order polynomials were fit to the stress-strain data. After polynomials were fit to the loading data, the maximum strain and stress of

the loading polynomial was recorded for further analysis, and the unloading polynomial was forced to pass through this point, thereby connecting the loading and unloading curves at the transition point. The curves were then numerically integrated using the trapezoidal rule with a resolution of 0.00001 strain. To calculate resilience, we divided work loading by work unloading. There was evidence of slipping during the unloading of one fascicle; for those data, only maximum strain and energy stored were analyzed. We also estimated the elastic modulus of the tendon fascicle by dividing the total change in stress by the total change in strain (E_{secant}). This value provides an average modulus of elasticity over the entire trial. Finally, to obtain the stress rate of loading, we divided the change in stress in the loading subset by ramp duration.

The data, which contain different numbers of replicates for each sample, were fit with a linear mixed-effects model in R using the nlme package (nlme version 3.1-128). To investigate the effect of stress rate on maximum strain we defined the fixed effect as *strain* ∼ *stress rate*. Due to the hierarchical nature of our dataset (n_observations = 175, n_treatments = 8, n_rats = 7), we defined replicate and rat ID as our two random effects. It is important to note that in our model, we define replicate as nested in rat ID (i.e., replicate 1 from one rat had no association with replicate 1 from another rat). We performed similar tests using identical random effects to determine the fixed effect of *energy storage*∼ *stress rate* as well as *resilience*∼ *stress rate*.

RESULTS

The average resulting strain across all trials was 0.57%. Given that all tendon fascicles were loaded to 4 MPa of stress, the average Young's Modulus of Elasticity (E_{secant}) of all trials was 691 ± 148 MPa (**Table 1**). The range of strain experienced by all fascicles was 0.458–0.874%.

The amount of maximum strain and stored energy in the tendon fascicles was negatively correlated with stress rate (**Figures 4**, **5**). These effects are demonstrated by the significantly negative relationships of strain (slope = -3.19×10^{-2}, $SE = 1.16 \times 10^{-2}$, $p = 0.007$) and energy storage (slope = -6.13×10^{-3}, $SE = 2.95 \times 10^{-3}$, $p = 0.040$) to stress rate (**Figure 5**). Compared to the slowest stress rates, at the fastest stress rates, maximum strain decreased from 0.637 to 0.614%. This was accompanied by a 3.48% decrease in energy storage. Conversely, increasing stress rate increased resilience (slope = $+ 4.98 \times 10^{-4}$, SE = 1.69×10^{-4}, $p = 0.004$).

TABLE 1 | The resulting strain rates from our testing protocol are greater than those used by other studies of rat tail tendon fascicles; but, our average measures of Elastic Modulus are comparable to previously reported data.

Study	Strain rate (%/s)	Modulus ± SD (MPa)
Current study	2.18–9.16	691 ± 148
Legerlotz et al., 2010	1	1000 ± 165
Screen et al., 2004	0.33	NA
Rigby et al., 1959	0.016–0.33	NA
Haraldsson et al., 2009	∼0.12	641 ± 30

DISCUSSION

The goal of this study was to determine whether rate of loading significantly influenced the mechanical behavior of tendon across a physiological range of loading. Our choice of loading rates were meant to span from some of the fastest loading rates that have been observed in vertebrates during movement to a relatively slow loading rate meant to represent the kind of loading that may occur in "ordinary" movements. We found that when tendon fascicles were loaded at faster rates, they stretched less, stored less energy, but were more resilient. These data suggest that loading rate does have the potential to influence tendon mechanical behavior *in vivo*, and thus muscle function via muscle-tendon mechanical interactions. The magnitude of the effect of loading rate was small, but, depending on factors such as the ratio of tendon length to muscle length in the muscle-tendon unit (MTU), small viscoelastic effects of tendon can affect muscle mechanical function.

Potential Benefits of the Rate-Sensitivity of Tendons

When loaded at fast rates, maximum strain and stored energy were reduced relative to slower stretches. Either of these has the potential to contribute to failure. Given that tendons are most susceptible to sports-related injury during high acceleration/deceleration exercise (Soldatis et al., 1997), the reduction of strain and stored energy at high loading rates has the potential to aid in reducing the chance of tendon failure. Studies of primate anterior cruciate ligaments that found that, at high rates of loading, ligaments failed at higher loads and greater elongations, suggesting some protective mechanism associated with high rates of loading (Noyes et al., 1974). At slow rates of loading, we found that maximum strain and energy storage increased. Given that the tendons that are used for elastic energy storage are loaded relatively slowly (Wainwright and Bennett, 1992; Lappin et al., 2006; Van Wassenbergh et al., 2008), the mechanical response of tendon fascicles complements their function at slow loading rates, allowing the tendon fascicles to stretch more and store more energy for a given amount of input stress. Tendon fascicles potentially limit stored energy when it is harmful and increase stored energy when it is of use; but, to determine the ability of tendons to modulate mechanical response, it is important to consider the size of these effects and their ability to influence organismal-level mechanics.

Estimating the Effects of Tendon Fascicle Viscoelasticity on MTU Mechanics

When a given amount of force is applied to a biological material (such as when a muscle stretches a tendon in a fixed-end contraction), lower stiffness results in higher strains. In the MTU, this results in comparatively more tendon stretch as stress rates decrease. In the context of muscle force generation, the sensitivity of tendon stretch to stress rate has the potential to affect muscle dynamics. For example, in a fixed-end contraction, a tendon that stretches to longer lengths would necessitate more muscle

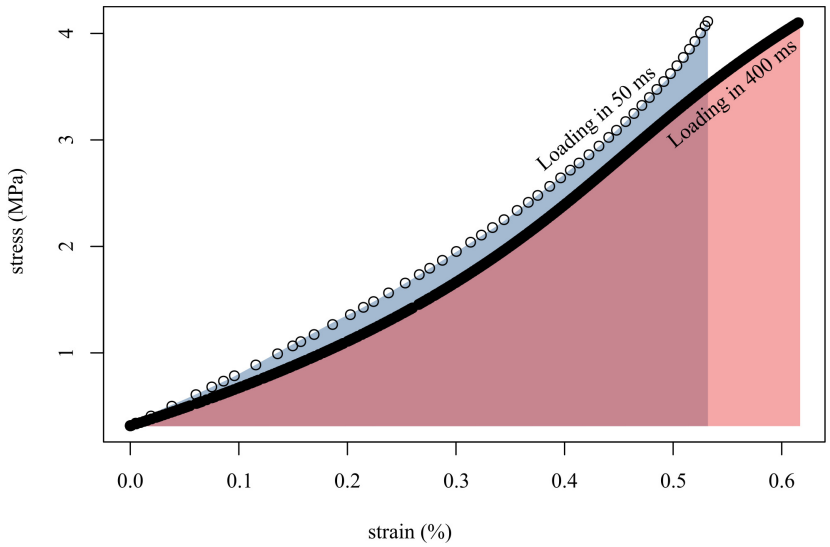

FIGURE 4 | Loading tendon fascicles quickly **(in 50 ms; open circles)** resulted in higher stiffness **(slope of open circles)** and lower final strain than when loading the same fascicles slowly **(in 400 ms; solid black line)**. This translated to more work done on the fascicle when loaded slowly **(red area)** than when loaded quickly **(gray area)**. Shown here are representative data from a single tendon fascicle.

fiber shortening. Changes to muscle fiber shortening strain or speed can influence muscle force output via the force-length and force-velocity behaviors of muscle. We found that tendon fascicle strain decreased 0.023% from 0.637 to 0.614% strain between our slowest and fastest experimental stress rates. On its own, this ∼4% change in tendon strain would seem to suggest a minor effect of loading rate on muscle fiber dynamics. However, depending on the dimensions of the muscle and tendon, the effect has the potential to be significant.

The influence of variation in tendon strain on muscle fiber strain will depend on the ratio of muscle fiber and tendon lengths. If we take as an example the human gastrocnemius we can calculate the maximum possible effect of loading rate that our data would suggest. Resting length values of 225 and 60 mm have been reported for the gastrocnemius tendon and fiber length (medial gastrocnemius) respectively (Maganaris and Paul, 2002; Lichtwark and Wilson, 2006), for a tendon/fiber length ratio of 3.6. Achilles tendon strains during running and maximum voluntary contractions have been reported as ∼4% (Maganaris and Paul, 2002; Lichtwark and Wilson, 2006). Our maximum expected change in strain from slow to fast loading of 4% would mean that loading rate has the potential to change achilles tendon strain *in vivo* by 0.04 × 0.04 = 0.0016, or 0.16%. Given the tendon/muscle fiber length ratio, this could alter fiber strain by 0.16 × 3.6 = 0.58%. Thus, loading rate under these conditions for this muscle seems unlikely to have a meaningful effect on muscle fiber dynamics.

Tendon to fiber length ratios vary among muscles, and for extreme systems the very small effects of loading rate observed here have the potential to significantly influence muscle fiber strain during contraction. In the extreme case of the flexor digitorum superficialis muscle in horses, which has been estimated to operate with a tendon that is 100 times longer than the resting length of the muscle (Biewener, 1998). The same calculation used above would yield, for this muscle, a 16% difference in muscle fiber strain when the tendon is loaded under slow vs. fast loading. Such a change would have significant influence on muscle fiber mechanical output via force-velocity and force-length behavior.

It seems unlikely that the measured significant increase in resilience in response to stress rate has an important impact on the mechanics of the MTU *in vivo*. At the slowest stress rates (where resilience is lowest), resilience remained relatively high at 87.23%, demonstrating that most elastic energy stored in the tendon fascicle was returned with little dissipation. Our least resilient tests fall slightly below the reported values for collagen found in mammalian tendon (90%; Pollock and Shadwick, 1994), for turkey hind limb tendons (90–94%; Matson et al., 2012), and for elastin fibers from bovine ligaments (90%; Aaron and Gosline, 1981). For comparison, cyclical strain tests that were conducted on the nuchal ligament of cows showed a decrease in resilience from 76 to 31% when increasing the frequency from 1 to 31 Hz (S. A. Wainwright et al., 1965). Our choice of unloading duration may have influenced our results to some extent (longer durations of unloading could lead to lower values of resilience); but, given that the duration of elastic recoil is 20–50 ms in jumping anurans (Astley and Roberts, 2014) and on the order of 20 ms in the tongue projection of chameleons (de Groot and van Leeuwen, 2004), our unloading duration of 100 ms provides conservative estimates. The relatively high values for resilience of rat tail tendon fascicles across all our experimental stress rates indicated that viscoelasticity did not play a major role in energy dissipation. Additionally, it is important to note that resilience in the tendon fascicles is lowest at slow rates of loading; therefore, even though our results suggest that more energy is stored in tendon fascicles with slow stress rates, less of that energy is recovered during

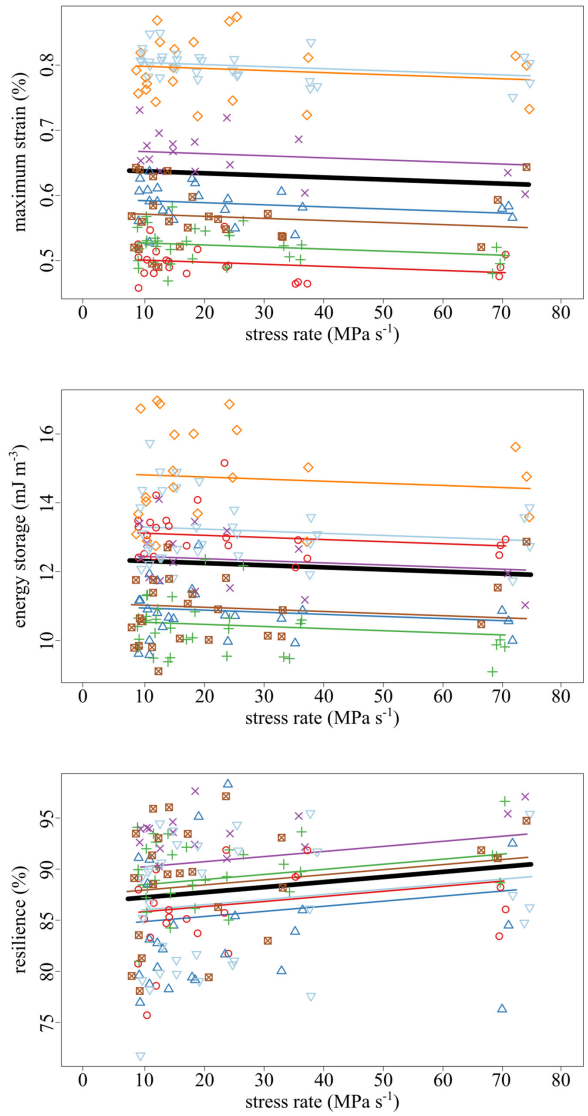

FIGURE 5 | As the stress rate of loading increased, both the maximum strain and strain energy storage decreased while the resilience increased. Data collected from the same tendon fascicle are represented by unique colors and symbols. A linear mixed-effects model was fit to the data to account for the hierarchy due to non-independence within replicates and samples. The thick black lines represent significant fixed effects of maximum strain ($p = 0.007$), energy storage ($p = 0.040$), and resilience ($p = 0.004$) regressed against stress rate. Thin lines represent within- individual relationships and are colored identically to the raw data to which they correspond.

unloading, potentially counteracting the energy storage benefits of stretching tendon slowly.

Limitations

Because tendons are hierarchical structures, it is possible for viscoelastic effects to emerge at various levels of organization; therefore, it is important to consider the benefits and limitations of investigating viscoelasticity solely at the fascicle level. A benefit of tendon fascicles studies is the abundance of rat tail tendon fascicle studies, thereby providing measurements that are directly comparable to our own (Hansen et al., 2002; Haraldsson et al., 2009; Legerlotz et al., 2010). For example, Elastic Moduli obtained in this study were consistent with previously published values. In all tests and across all rates of loading, the average value for E_{secant} was 691.18 ± 148.88 MPa, which was consistent with previously published calculations for Young's modulus of elasticity (**Table 1**).

Although not perfectly elliptical, the cross-sectional area of a tendon fascicle in rat tail tendons is generally simpler and more uniform in shape than that of whole tendon. The simplified morphology of tendon fascicles reduces potential errors in the calculation of stress. The major drawback of focusing on tendon fascicles, however, is that our results may not necessarily reflect mechanics of the whole tendon. For example, the viscoelastic behavior of horse tendons has been attributed to the interfascicular matrix (Thorpe et al., 2015, 2016, 2012). Additionally, the viscoelastic effects at play during stress-relaxation of rat tail tendons are highly dependent on the effects of fibers sliding past each other (Screen, 2008; Gupta et al., 2010). These effects are completely ignored when testing solely at the fascicle level. Although some studies have shown that the fascicles of some tendons likely function as independent structures with little to no interaction occurring between fascicles (Haraldsson et al., 2008), it is important to remember that any mechanical interaction between tendon fascicles is not captured in the present study.

The applied stresses and measured strains in this study were much lower than those typical of most studies of whole tendons. Because our mechanical tests required repeatable trials per sample and multiple replicates without failure, we used conservative upper bounds for maximum stress. Previous studies have found that applying up to 4 MPa of stress during preconditioning did not alter rat tail tendon fascicle mechanics in subsequent trials (Haraldsson et al., 2005); therefore, we chose to apply no more than 4 MPa of stress during all of our trials. As a result, the maximum strain achieved across all trials was less than 1% Additionally, in our preliminary tests, we were not able to exceed 1.5% strain without failure. Although previous studies have reported up to 4% (Rigby et al., 1959) strain and 12 MPa of stress before indication of failure of tendon fascicles (Haraldsson et al., 2009), we were not able to replicate these results. It is likely that our method of testing caused concentrations of stress and/or strain in the tendon fascicle near the clamp, resulting in failure at much lower strains and stresses than previously reported. Despite the possible stress concentrations in our samples, when stress did not exceed 4 MPa, our data showed no indication of plastic deformation or failure.

It is also important to note that because we restricted our tests to low stress values, we could not directly measure the effects of viscoelasticity at the normal operating levels of strain. In reality, high rates of stress lead to substantial tendon strain within short durations, however, our data are only representative of the "toe region" of rat tail tendon stress-strain curves, which occurs within 1–2% strain of the whole tendon (Hansen et al., 2002; Screen et al., 2004). Despite our maximum strain falling slightly below this range, most reported estimates of strain (including the measurements of the "toe region" mentioned above) are

made using grip-to-grip distance whereas our visual method measured strain mid-substance. Studies have shown that strain measured via grip-to-grip distances is consistently higher than strain measured mid-substance (Haraldsson et al., 2005). Indeed, the values of maximum strain we measured using our optical method was on average 0.278% less than the strain measured using grip-to-grip distances (see **Supplementary Figure S1**). A major design choice in this study was to avoid failure by applying small stresses to the tendon fascicles; however, given that previous work on mice tail tendon fascicles showed small rate effects on elastic modulus but large sensitivity to yield stress (Robinson et al., 2004), future work should test viscoelasticity at higher stresses and strains.

CONCLUSION

Despite the significant effects of stress rate on maximum strain, energy storage, and resilience, these effects were not large enough to dominate non-specialized muscle-tendon mechanics at rates relevant to transient loading events. These data complement previous studies that demonstrated long-range rate dependence via tensile creep and stress-relaxation tests in collagenous tissues. In these cases, the effect of rate has large effects on the mechanical properties of these tissues. It is likely then that the importance of viscoelastic effects gradually increases with the duration of loading. At the durations of tendon loading that are likely to occur during landing and power amplification, however, the present results suggest that viscoelasticity can be largely ignored.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

Samples were collected from animals euthanized for unrelated studies. All animal use for these unrelated studies was approved by the Brown University Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

MR designed the experiment and collected and analyzed the data. TR helped design the experimental rig and provided guidance. MR and TR both contributed to the writing and editing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00255/full#supplementary-material

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Mechanical and Material Tendon Properties in Patients With Proximal Patellar Tendinopathy

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Introduction: The effect of chronic patellar tendinopathy on tissue function and integrity is currently unclear and underinvestigated. The aim of this cohort comparison was to examine morphological, material, and mechanical properties of the patellar tendon and to extend earlier findings by measuring the ability to store and return elastic energy in symptomatic tendons.

Methods: Seventeen patients with chronic (>3 months, VISA-P < 80), inferior pole patellar tendinopathy (24 ± 4 years; male = 12, female = 5) were carefully matched to controls (25 ± 3 years) for training status, pattern, and history of loading of the patellar tendon. Individual knee extension force, patellar tendon stiffness, stress, strain, Young's modulus, hysteresis, and energy storage capacity, were obtained with combined dynamometry, ultrasonography, magnetic resonance imaging, and electromyography.

Results: Anthropometric parameters did not differ between groups. VISA-P scores ranged from 28 to 78 points, and symptoms had lasted from 10 to 120 months before testing. Tendon proximal cross-sectional area was 61% larger in the patellar tendinopathy group than in the control group. There were no differences between groups in maximal voluntary isometric knee extension torque ($p = 0.216$; $d < -0.31$) nor in tensile tendon force produced during isometric ramp contractions ($p = 0.185$; $d < -0.34$). Similarly, tendon strain ($p = 0.634$; $d < 0.12$), hysteresis ($p = 0.461$; $d < 0.18$), and strain energy storage ($p = 0.656$; $d < 0.36$) did not differ between groups. However, patellar tendon stiffness (−19%; $p = 0.007$; $d < -0.74$), stress (−27%; $p < 0.002$; $d < -0.90$) and Young's modulus (−32%; $p = 0.001$; $d < -0.94$) were significantly lower in tendinopathic patients compared to healthy controls.

Discussion: In this study, we observed lower stiffness in affected tendons. However, despite the substantial structural and histological changes occurring with tendinopathy, the tendon capacity to store and dissipate energy did not differ significantly.

Keywords: patellar tendinopathy, VISA-P, tendon viscoelastic properties, tissue function, tissue integrity

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INTRODUCTION

Tendinopathy is a multifactorial tissue disorder (Riley, 2005) responsible for almost every second sport- or occupational-related injury (Jung et al., 2009) and impedes physical function and performance (Maffulli et al., 2003; Lian et al., 2005; Malliaras and Cook, 2006). The precise pathogenesis remains vague, but the disease is often described as overload injury, caused by a mismatch between the functional demand and the adaptational rate of tendinous tissue (Riley, 2004; Magnusson et al., 2010). Degenerated tissue is characterized by essential alterations in the extra-cellular matrix content, including a proliferation of hydrophilic macromolecules (e.g., proteoglycans, glycosaminoglycans; Riley, 2005), proportional changes of type I toward type III collagen (Maffulli et al., 2000), increased crosslinking concentration (Kongsgaard et al., 2009) or lower fibril density (Kongsgaard et al., 2010). Despite some of these changes in tissue composition being able to affect tendon viscoelastic properties, the global effect of tendinopathy on the mechanical properties of the patellar tendon remains elusive (Obst et al., 2018).

Tendons play an essential role by enabling the utilization of elastic energy toward efficient muscle power output and buffering rapid stretch of muscle fibers (Roberts and Azizi, 2011). Muscular function and integrity are thus closely linked to tendon mechanical properties or change thereof. Tendon mechanical properties and their change are thus of paramount importance for muscular function and integrity. While growing evidence suggests that tendinopathic Achilles tendons present a lower stiffness than healthy controls, the studies on patellar tendon have thus far been discordant. *In vivo* measures on tendinopathic patellar tendons currently indicate either a deficit (−20%, Helland et al., 2013) or did not find evidence for differences in tendon stiffness (Kongsgaard et al., 2010; Couppé et al., 2013; Lee et al., 2017) when comparing affected and asymptomatic, healthy sides. Likewise, reports indicate that patellar tendon strain and Young's modulus are either lower, higher, or similar in affected patellar tendons (Obst et al., 2018).

These inconsistent findings have been ascribed to differences in the severity of the disease, and to the challenges to assess tendon mechanical properties *in vivo* (Lee et al., 2017). Given the few studies (n = 4, to date) on this topic, additional research is required to elucidate whether tendinopathy alters the stiffness of the patellar tendon, as suggested by alterations in extracellular matrix composition and by certain studies on patellar (Helland et al., 2013) and Achilles tendons (Wang et al., 2012; Chimenti et al., 2014; Chang and Kulig, 2015) or whether this condition has no effect on the mechanical properties of the patellar tendon (Kongsgaard et al., 2010; Couppé et al., 2013; Lee et al., 2017).

Furthermore, although tensile stiffness has received all the attention of studies assessing the impact of tendinopathy on tendon mechanical properties, there is to date no information regarding tendon elastic hysteresis. The energy dissipation (∼9% in healthy tendons; Bennett et al., 1986) occurring during recoil affects the amount of energy recovered from tendon stretch and, importantly, may be linked to the tissue susceptibility to fatigue damage (Maganaris et al., 2008; Farris et al., 2011; Lichtwark et al., 2013; Herod and Veres, 2017). The current lack of information about tendon hysteresis seems mainly due to the methodological difficulty to test this parameter *in vivo* (Finni et al., 2013). However, over the past few years, some research groups were able to propose reliable methods to measure tendon hysteresis *in vivo* (Kubo, 2004; Peltonen et al., 2012, 2013; Stenroth et al., 2012; Fouré et al., 2013; Wiesinger et al., 2017). Some of these studies indicated that tendon hysteresis levels are related to loading patterns (Reeves et al., 2003; Kubo, 2004; Fouré et al., 2010) in a way that could not be predicted from other biomechanical parameters (Wiesinger et al., 2017). Hence, despite the known plasticity of tendon hysteresis with loading conditions and, possibly, with changes in tendon composition, there is currently an unmet clinical need to characterize the influence of tendinopathy on this parameter.

In this study, we propose to conduct an exhaustive assessment of patellar tendon properties in tendinopathic patients and in matched controls by including for the first time tendon hysteresis measurements. A secondary aim was to extend the body of literature about stiffness of tendinopathic tendons.

We predicted that tendinopathic patellar tendons to exhibit greater cross-sectional area (CSA) of the affected region, but a decreased elastic modulus when compared to asymptomatic, healthy control tendons. Based on the limited evidence, we hypothesized that the chronic patellar tendinopathy of the inferior pole would not meaningfully effect tensile tissue strain and stiffness. Given the complexity of histological changes associated with tendinopathy and in the absence of previous data to specify this hypothesis, the purpose of this exploratory study was also to determine whether hysteresis would be lower or higher with this disorder.

MATERIALS AND METHODS

Subjects

Seventeen patients with chronic (>3 months; Leadbetter, 1992) patellar tendinopathy (24 ± 4 years, male = 12; female 5) and 17 strictly matched healthy controls (25 ± 3 years, male = 12; female 5) volunteered to participate in this cohort comparison study (**Supplementary Figure S1**). Screening of eligibility was confirmed by a specialist for Physical Medicine and Rehabilitation (J.H) at the Institute of Physical Medicine and Rehabilitation of the Paracelsus Medical University Salzburg. Examination of tendinopathy included persistent but aggravated pain during or after weight-bearing activity, impaired functional performance (VISA-P ≤ 80, Visentini et al., 1998; Zwerver et al., 2011) and palpation tenderness around the affected tissue region. Other or additional knee joint injuries were largely excluded using a comprehensive manual clinical examination (e.g., pivot shift test, Steinmann II test, Lachman test). Longitudinally, ultrasonography and magnetic resonance images verified anterior-posterior tendon thickening of at least 1 mm compared with mid-tendon level (Couppé et al., 2013) irregular tendon structure, hypoechoic area and several cases of neovascularization (n = 13, male = 10; female 3)

and/or non-tendon-like phenotypes, including bone formation or heterotopic ossification (n = 7, male = 6; female 1) at the proximal tendon region only (Black et al., 2004). The present protocol focused on proximal patellar tendinopathy to allow for interstudy comparisons (Kongsgaard et al., 2010; Couppé et al., 2013; Helland et al., 2013; Lee et al., 2017). Non-symptomatic tendons were free of any of these signs of degeneration, and the pain-free group reported no previous history of any known tendon pain. Further exclusion criteria included self-reports of neuromuscular, cardiovascular or respiratory disorders, diabetes, disclosure of anabolic drug abuse, use of oral anticoagulants, any knee surgery, or injections in or along the patellar tendon in the preceding 12 months, Osgood-Schlatter disease or other knee pathologies that potentially impede maximal muscle contraction. Besides, subjects with oral confirmation of claustrophobia or incorporated metallic foreign implants were excluded based on magnetic resonance imaging (MRI). Recruitments were consecutive via local newspaper advertisements, social media, and verbal contacts between March 2017 and August 2019. Purposes, benefits, and risks of testing procedures were given before obtaining written informed consent. The study is part of an ongoing clinical project (German register for clinical trials DRKS00011338) and was approved by the Local Research Ethics Committee (415-E/2012/11-2016).

Experimental Design

Data of each individual were collected within 2 weeks by the same experienced investigators (H-P.W, F.R) at the Department of Sport and Exercise Science. In patients suffering from chronic patellar tendinopathy, lower leg strength and tendon properties were estimated in the leg of the pathology, or that leg of the most severely affected tissue disorder. In the healthy matched case-control subject, leg selection was based on leg dominance (Büsch et al., 2009) of the paired subject with tendinopathy. All subjects confirmed a 24 h absence of vigorous activities (Shalabi et al., 2004; Boesen et al., 2006) received detailed instructions, contemporaneous visual feedback, and conducted at least two exercise-specific familiarization trials prior to each testing. Investigators provided strong verbal

TABLE 1 | Anthropometric characteristics, training status, and degree of patellar tendon symptoms.

	Tendinopathy	Controls	t-Value$_{(16)}$	Difference BCa 95% CI	P-Value	d-Value
Age (years)	23.7 ± 1.00	25.0 ± 0.66	1.64	[−0.17, 2.45]	0.119	0.40
Body mass (kg)	76.9 ± 3.3	74.9 ± 3.3	−1.99	[−4.65, 0.18]	0.063	0.44
Body height (cm)	179.3 ± 2.10	178.9 ± 2.41	−1.89	[−4.03, 3.84]	0.925	0.05
BMI (kg·m^{-2})	23.2 ± 0.69	23.8 ± 0.80	−0.10	[−1.56, 0.17]	0.076	0.34
Training experience (years)$^{\#}$	8.9 ± 1.27	9.6 ± 1.57	1.83	[0.32, 3.86]	0.091	0.44
Training (hours/week)$^{\#}$	7.4 ± 1.00	5.6 ± 0.82	−1.17	[−2.38, 0.38]	0.265	0.28
Symptom duration (months)	38.3 ± 8.18					
VISA-P score	56.6 ± 3.49					
VAS	7.1 ± 0.40					

Values are expressed as mean ± standard error of the mean. BMI, body mass index; VISA-P, Victorian Institute of Sport Assessment-Patellar tendon; VAS, visual analog scale for pain; $^{\#}n$ = 15 because of two non-athletes in each group. Bias-corrected and accelerated (BCa) 95 percent bootstrap confidence interval.

TABLE 2 | Knee extension maximal torque and patellar tendon properties.

	Tendinopathy	Controls	t-Value$_{(16)}$	Difference BCa 95% CI	P-Value	d-Value
Isometric torque (N·m)	204 ± 14	226 ± 19	−1.29	−22 [−57, 10]	0.216	−0.31
Tendon dimensions						
Tendon length (mm)	49.6 ± 1.7	46.7 ± 1.6	0.85	0.2 [−0.2, 0.6]	0.410	0.21
Tendon mean CSA (mm^2)	148 ± 7.8***	111 ± 5.5	−4.50	37 [22, 52]	<0.001	−1.09
Ramp contraction						
Peak force (N)	5271 ± 287	5909 ± 534	−1.39	−638 [−1745, 302]	0.185	−0.34
Stress (MPa)	35.8 ± 1.4**	53.9 ± 4.7	−3.70	−18.1 [−28.5, −10.1]	0.002	−0.90
Strain (%)	8.8 ± 0.4	8.6 ± 0.4	0.49	0.3 [−0.9, 1.3]	0.634	0.12
Stiffness$_{max}$ (N·mm^{-1})	2029 ± 170**	2617 ± 210	−3.07	−587 [−939, −234]	0.007	−0.74
Stiffness$_{com}$ (N·mm^{-1})	1745 ± 146	1887 ± 75	−0.80	−142 [−430, 210]	0.435	−0.19
Young's modulus$_{max}$ (GPa)	0.69 ± 0.05**	1.10 ± 0.09	−3.88	−0.41 [−0.63, −0.23]	0.001	−0.94
Young's modulus$_{com}$ (GPa)	0.58 ± 0.04**	0.85 ± 0.06	−3.49	−0.27 [−0.43, −0.12]	0.003	−0.85
Triangular loading ramps (Figures 3A,B)						
Hysteresis (%)	18.6 ± 1.7	17.1 ± 1.0	0.76	1.4 [−1.9, 4.8]	0.461	0.18
Strain energy (J)	7.0 ± 0.5	8.5 ± 1.2	−1.48	−1.5 [−3.7, 0.3]	0.656	0.36

*Values are expressed as mean ± standard error of the mean. Bias-corrected and accelerated (BCa) 95 percent bootstrap confidence interval. **$p < 0.01$, ***$p < 0.001$ compared with healthy control patellar tendons.*

encouragement throughout attempts with resting periods lasting for one minute between tests and half a minute between trials of the same test. Questionnaires were answered without investigator assistance, and all offline analyses were executed in a subject-blinded fashion.

Patellar Tendon Morphology

Patellar tendon resting length and regional tendon CSA were assessed from sagittal and axial plan magnetic resonance imaging, respectively (3T-Achieva, Philips Healthcare, Eindhoven, Netherlands; lower extremity coil and the following parameters: TR/TE 682/20ms, FOV 100, matrix 528 × 528, slice thickness 3.0 mm, space 3.3 mm), and analyzed offline using an image-processing program (ImageJ, Rasband, W.S., National Institutes of Health, Bethesda, MD, United States)[1]. Tissue length was defined as the distance between the tibial insertion and the apex of the patella. Mean patellar tendon CSA was obtained as the average of three separate measures performed at the proximal (pCSA) and distal (dCSA) insertion and at tendon mid-length (mCSA). Analyses were conducted by the same orthopaedist (YS). The reliability of MRI-based measurements of patellar tendon CSA has been reported in previous publications (Seynnes et al., 2009; Stenroth et al., 2019).

Maximal Voluntary Contraction

Lower leg strength and tendon properties measurements were preceded by a warm-up routine consisting of 10-min of cycling at 1.5 $W \cdot kg^{-1}$ at a cadence of ∼70 rpm on a stationary ergometer (Heinz Kettler GmbH and Co. KG, Ense-Parsit, Germany). The rotational center of the isokinetic dynamometer arm (IsoMed 2000 D&R Ferstl GmbH, Hemau, Germany) was carefully aligned with the rotation axis of the knee joint. Subjects sliding was minimized by means of firm securing with adjustable shoulder pads, hip and footplate straps. Hip and knee joint angles were set to 75° and 90° (0° corresponding to full extension) throughout all test procedures (Dirnberger et al., 2012).

Knee extensor and flexor muscle torques were obtained from two maximal isometric contractions (MIVC), with a gradual but continuous ∼2-s build-up period and a 3-s plateau. The trial yielding the highest peak torque was retained for analysis. Three additional MIVCs were performed at a rate of 50 $Nm \cdot s^{-1}$ with visual feedback to test patellar tendon properties (see below). Tensile tendon force was calculated offline as the difference between the net extension torque and the torque produced by antagonist's muscles, divided by the patellar tendon moment arm, which was based on a function of upper segment leg length (Visser et al., 1990). Antagonist muscle torque was estimated from surface electromyographic (sEMG) activity, by assuming linearity between sEMG and torque produced during knee flexion and extension (Magnusson et al., 2001). To this end, surface electrodes (Ag/AgCL; 120 dB, Input impedance: 1200 GOhm; 10 mm diameter, 22 mm spacing, Biovision, Wehrheim, Germany) were apposed on the biceps femoris and semitendinosus muscles. Raw sEMG signal was filtered offline using a second-order Butterworth filter with a cut off frequency of 10, and 300 Hz. Maximal sEMG amplitude was quantified when the hamstrings acted as agonists as the root mean square of the signal over a 0.5-s period around the peak torque of knee flexion (Wiesinger et al., 2016). This technique has shown high interday reliability (Reeves et al., 2004).

[1]http://imagej.nih.gov/ij/

Patellar Tendon Properties

Patellar tendon stiffness, stress, strain, and Young's modulus were obtained from isometric ramp contractions up to the maximal level of voluntary exertion. Hysteresis and strain energy were acquired from symmetrical loading and unloading ramps with a fixed peak load of 80% of individual MIVC torque levels ($MIVC_{80\%}$). The targeted and produced torque were displayed on visual feedback, and several practice trials were performed for familiarization and tendon preconditioning (Maganaris, 2003) before each test. The reliability of these techniques has been demonstrated previously (Kösters et al., 2014; Wiesinger et al., 2016, 2017).

Tendinous tissue elongation was captured at 29 Hz by placing an ultrasound probe (linear array transducer 5 cm, LA523, 10–15 MHz transducer, MyLab25, Esaote, Genoa, Italy) sagittal over the patellar tendon. Scans were then analyzed offline by an experienced investigator (H-P.W) as the displacement between the patella apex and the tibial anterosuperior aspect using a semi-automatic video analysis software (Tracker 4.87, physlets.org/tracker/). Dynamometer signals and sEMG data were collected at a sampling rate of 2000 Hz, and all data records were synchronized from sending an electrical pulse grabbed simultaneously by the dynamometer and ultrasound system (Matlab, version R2017b; The MathWorks Inc., Natick, MA, United States). Force-deformation and stress-strain slopes were fitted using a constrained least-square function (Wiesinger et al., 2017). Patellar tendon stiffness (Δforce/Δdeformation) and Young's modulus (Δstress/Δstrain) were calculated from the highest individual and common 10% force interval or stress interval, respectively. Stress (tensile force/CSA) and strain (Δtendon length/tendon length at rest) values reflect the highest tissue force or strain. Hysteresis values were determined as the percent difference between the areas under the ascending and descending phases of the stress-strain curves obtained during $MIVC_{80\%}$ trials. Strain energy was calculated as the area under the ascending stress-strain loading curves during the same tests. Each test condition consisted of a minimum of five trials, but load-deformation data were only retained if the torque did not deviate from individual MIVC by more than 5%, and the curve-fitting coefficient of determination R^2 exceeded 0.90. Where possible, three trials were averaged, however, in some cases only two trials achieved the above requirements.

Statistics

Statistical analyses were performed using IBM SPSS Statistics V.26.0 (SPSS Inc., Chicago, IL, United States). Two-tailed paired-samples *t*-tests were computed to test for differences between patients and healthy controls in all investigated

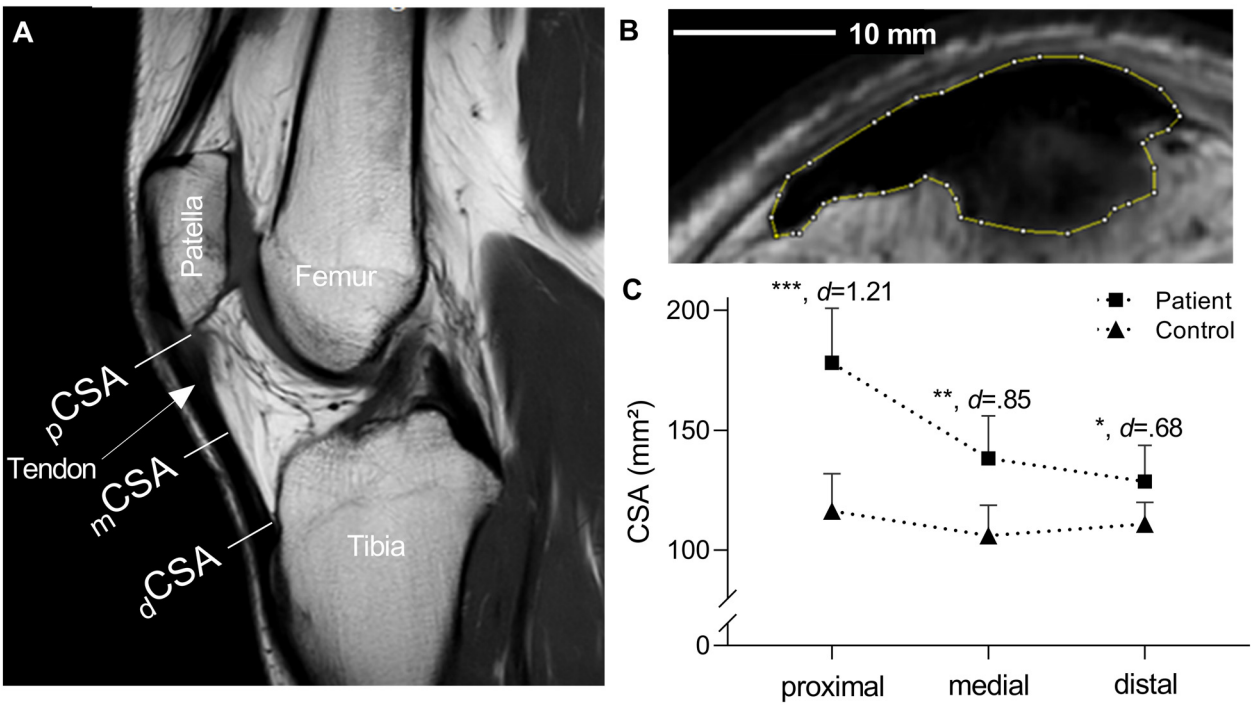

FIGURE 1 | Regional patellar tendon cross-sectional areas (CSA). **(A)** Sagittal magnetic resonance (MRI) scan of the patellar tendon and denoted positions of CSA measurements. **(B)** Segmented, axial MRI of patellar tendon proximal-region in one representative patient with tendinopathy. **(C)** Between-group comparisons of patellar tendon CSA. Values are mean ± 95% confidence limits. $_{p}$CSA, cross-sectional area proximal; $_{m}$CSA, cross-sectional area medial; $_{d}$CSA, cross-sectional area distal; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with healthy control patellar tendons.

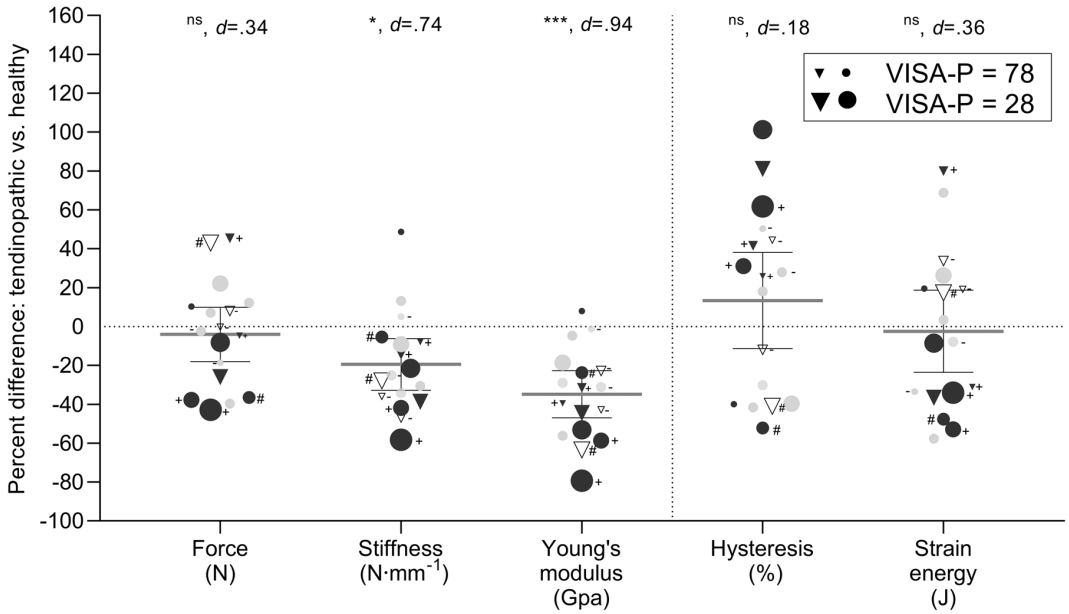

FIGURE 2 | Percentage differences in tendon tensile force, mechanical, and material properties between chronic tendinopathic and healthy patellar tendon properties. Mechanical and material properties were evaluated at a common force level of each individual matched pair. Scatter plots with mean ± 95% confidence limits sizes as an approximate representation of clinical severity are shown (see figure legend). Circles indicate male and triangle female subjects. Black filled symbols indicate cyclic loading (long-distance running, football, American football), gray filled symbols spring-like loadings (volleyball, team handball), and white symbols skiers and recreational actives (no specific sport activity). The symbol label refers to vascularity and calcification, with no label means vascularization only, # calcification only, + both, and – nothing. ns, non-significant, $^{*}p < 0.05$, $^{***}p < 0.001$ compared with intact patellar tendons.

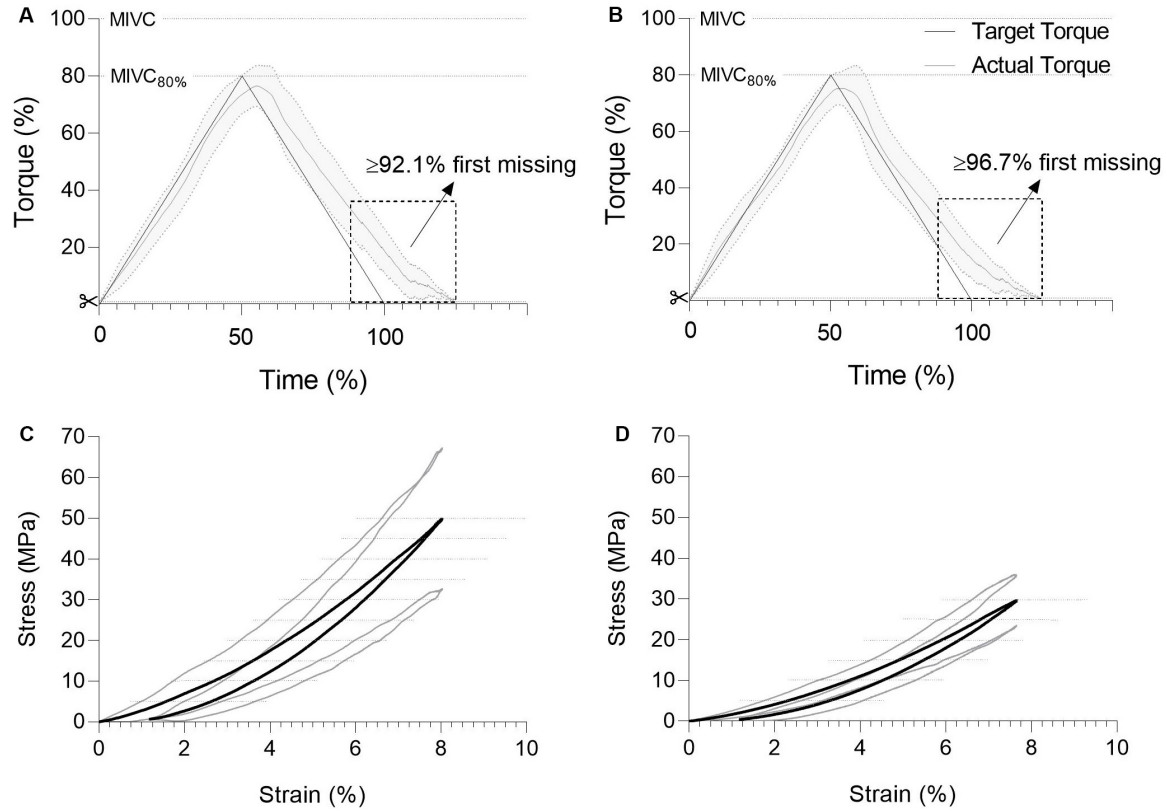

FIGURE 3 | Between-group comparison of patellar tendon viscoelastic properties. Plots show the average torques via triangular loading ramp of the healthy **(A)**, and impaired subjects **(B)** plotted against time, and the associated mean stress-strain curves of the intact **(C)** and tendinopathic tendons **(D)**. MIVC, maximal isometric voluntary contraction. Values are means ± SD [gray area **(A,B)** and additional gray lines **(C,D)** of all subjects].

parameters. The normality of the standardized residuals was analyzed conducting a Shapiro-Wilk test, and the Wilcoxon signed-rank test was applied in case of a non-normal residual distribution. The confidence interval for the mean difference was obtained by using a 95% bootstrap method with bias-corrected and accelerated (BCa). The effect size (d) was defined as small for $d > 0.2$, medium for $d > 0.5$, and large for $d > 0.8$ (Cohen, 1988). Pearson correlation coefficients were used to examine the relationship between the differences in the variables of the tendinopathic individuals and matched controls to the severity of the symptoms and functional limitations (VISA-P score). Figures were created using the GraphPad Prism 8.4.2 (GraphPad Software Inc., La Jolla, United States). Unless otherwise stated, results were expressed as mean ± standard deviation (SD). The level of significance was set a $p = 0.05$.

RESULTS

Subject Characteristics

Since athletic activity background and volume were used as matching criteria, all pairs of case and control practiced the same sport, at the same national or international level, for a similar number of years and according to comparable weekly volumes. Patients and controls had thus loaded their patellar tendon according to comparable functional requirements. Athletic activity included football ($n = 12$), volleyball ($n = 8$), team handball ($n = 4$), skiing ($n = 4$), high level of long-distance running ($n = 2$), American football ($n = 2$), and recreational activities ($n = 2$). In line with activity background and volume, anthropometric parameters did not differ between groups. VISA-P and VAS scores ranged from 28 to 78 and 3.0 to 9.5 points, respectively, and symptoms had lasted from 10 to 120 months before the examination (**Table 1**). The symptom time duration was not related to percent differences of matched pairs in tendon proximal CSA ($r^2 = 0.12$, $p = 0.171$), stiffness measured to individual ($r^2 = 0.01$, $p = 0.690$) or highest common force levels ($r^2 = 0.04$, $p = 0.452$), Young's modulus ($r^2 < -0.01$, $p = 0.647$), hysteresis ($r^2 = 0.05$, $p = 0.368$), or VISA-P scores ($r^2 < -0.03$, $p = 0.527$).

Muscle Strength and Tendon Properties

Maximal knee extension torque and tendon morphological, mechanical, and material properties are reported in **Table 2**.

Patellar tendon length was similar between groups, but mean CSA was significantly increased in the tendinopathic group.

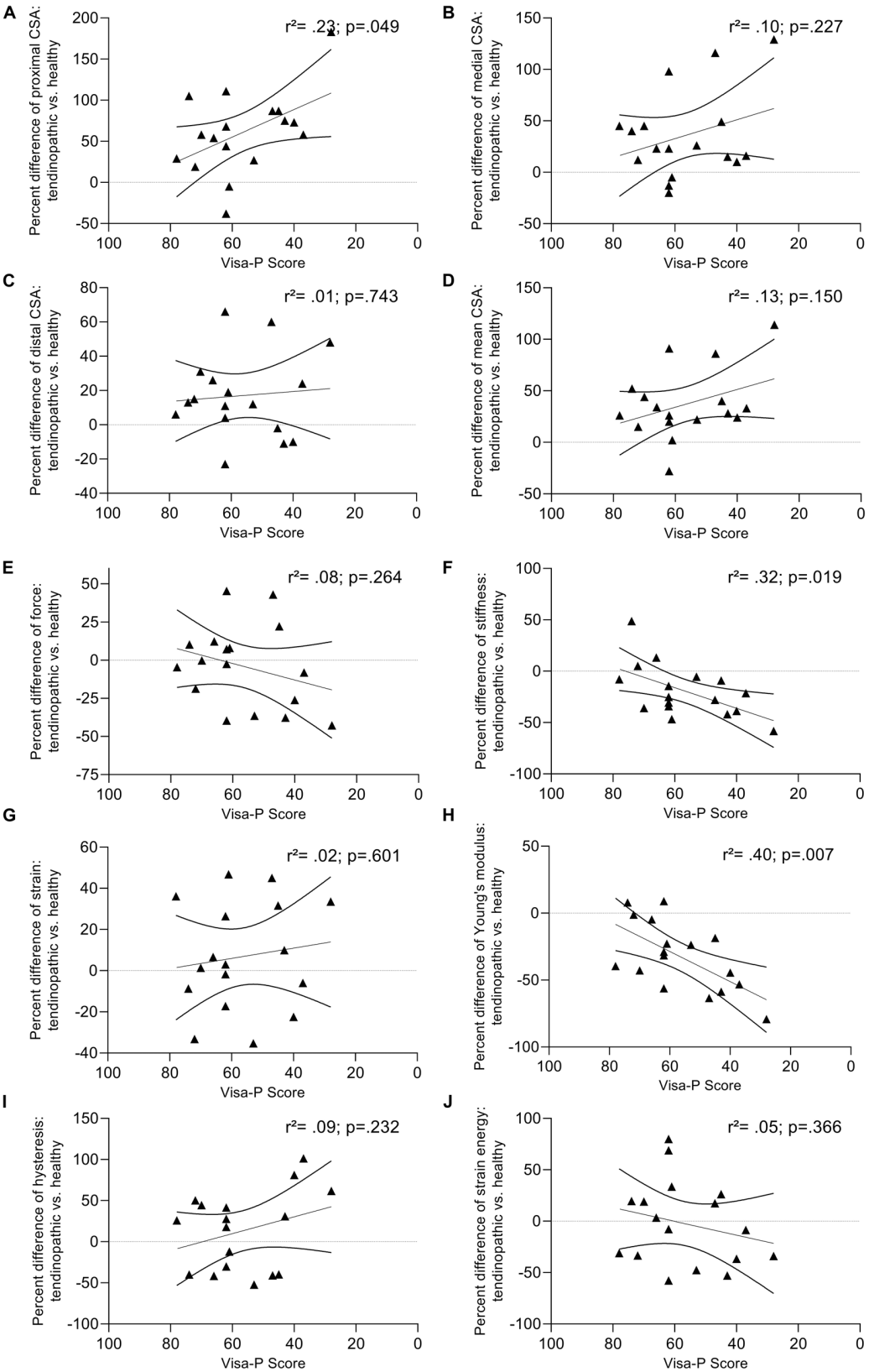

FIGURE 4 | Differences in muscle strength and tendon properties of affected individuals and healthy controls plotted against the scores of the VISA-P questionnaire. Between-group comparison of patellar tendon dimension **(A–D)**, tendon tensile force **(E)**, stiffness **(F)**, strain **(G)**, Young's modulus **(H)**, hysteresis **(I)**, and strain energy **(J)**. Lines depict the least-squares regression and their 95% confidence limits.

Region-specific comparison of CSA indicated that the pathology caused a significant tendon swellings at the proximal (+61%, BCa 95% [32.6, 86.4], $t_{(16)} = 4.98$, $p < 0.001$, $d = 1.21$), medial (+36%, BCa 95% [16.5, 48.7], $t_{(16)} = 3.51$, $p = 0.003$, $d = 0.85$), and distal tissue site (+17%, BCa 95% [5.9, 30.6], $t_{(16)} = 2.80$, $p = 0.013$, $d = 0.68$) (**Figure 1**).

There was no difference in maximal knee extension torque or patellar tendon peak force when testing tendon mechanical properties. Patellar tendon stiffness did not differ between groups when measured at the greatest common force level. However, when measured at the individual highest force level, patellar tendon stiffness was lower (−18%) in the tendinopathic group compared to controls. Additionally, patellar tendon stress (−27%) and absolute and relative Young's modulus (−32 and −33%) were significantly lower in tendinopathic patients compared to controls, but there was no difference in tendon strain (**Table 2** and **Figure 2**). Patellar tendon hysteresis and elastic energy storage seem unaffected by tendinopathy (**Table 2** and **Figure 3**). Furthermore, there was a significant correlation between the impaired patellar tendon properties and the clinical severity of symptoms represented by the scores of the VISA-P questionnaire for proximal tendon CSA, tissue stiffness and Young's modulus (**Figure 4**).

DISCUSSION

Despite the substantial structural and histological changes occurring with tendinopathy (Magnusson et al., 2010) energy dissipation remained unchanged in the affected tendons included in this study. Additionally, differences in tendon stiffness between tendinopathic and healthy tendons were only seen at maximal individual force and not at common force level.

Patient reports of VISA-P and VAS scores (**Table 1**) are consistent with similar cross-comparison studies of individuals with chronically affected tendons (Couppé et al., 2013; Lee et al., 2017) and demonstrate the severe functional impairment of the included cohort. However, in contrast to a previous observation (Couppé et al., 2013; Helland et al., 2013) affected patellar tendons were larger near the affected region (CSA +61%), but also in tissue areas with no apparent hypoechoic areas, neovascularization or presence of worse-aligned collagen structure (**Figure 1**). We can neither elucidate mechanisms nor discern whether the observed tissue thickening is the cause or the consequence of the pathological processes, but these findings suggest that tendinopathy also affects tissue sites that appear asymptomatic on MRI and ultrasound images.

Consistent with previous observations, the greater tendon size was not reflected in higher stiffness values, but chronically diseased tendons exhibited a lower stiffness in high loading conditions ($\text{stiffness}_{\text{max}}$) and a lower Young's modulus. Contrasting differences in tissue size and stiffness between tendinopathic and healthy patellar tendons had been previously observed by Helland et al. (2013) (proximal CSA +19%; stiffness −20%), with a similar trend in the findings from Kongsgaard et al. (2010) (stiffness -9%). Other studies have, however, not found any difference between the tendon stiffness of affected and healthy control patellar tendons (Couppé et al., 2013) or even +22% higher values, though not statistically significant (Lee et al., 2017). Taken together, the different outcome obtained when measuring stiffness at individual or common maximal force level and these aforementioned studies suggest that part of the discrepancy is attributable to methodological factors. Previous studies assessing patellar tendon mechanical properties have typically measured stiffness at a submaximal force level, corresponding to the highest force common to all subjects (Kongsgaard et al., 2010; Couppé et al., 2013; Helland et al., 2013; Lee et al., 2017). While this standardizing approach is appropriate for homogenous sample populations, stiffness may be inadequately measured outside the linear portion of the force-deformation relationship in groups with broader variations in force (Seynnes et al., 2015). In our sample population, tendon force ranged from 3063 to 10,963 N, indicating that the stiffness of the stronger subjects was measured in the toe region. In contrast, another study found a lower stiffness in affected vs. healthy patellar tendons using both calculation methods (Helland et al., 2013). However, the force level common to all subjects in that study (∼70% of control max force) was substantially higher than in the current one (∼55% of control max force), supporting the idea of poor suitability of this approach for our data. Hence, although stiffness values may be influenced by the force level at which they were calculated (O'brien et al., 2010) the advantage conferred by the consideration of force levels closest to the elastic portion of the deformation curve seems to prevail over the methodological inconsistency described above. For this reason, we contend that patellar tendon stiffness is reduced with tendinopathy. Interestingly, however, the lower stiffness measured at maximal individual force in the symptomatic tendons was not reflected by any difference in tendon maximal strain. This observation is congruent with the findings of other studies (Helland et al., 2013) and may reflect the different effects of tendon structure and composition on tissue behavior at various levels of tensile load.

Conversely, the similar tissue hysteresis and energy storage capacity of both patients and controls suggest that pathological patellar tendons retain a similar ability to use elastic energy. High elastic modulus, large strains, and relatively low hysteresis (∼9%) are common features of various mammalian tendons (Ker, 1981; Bennett et al., 1986; Shadwick, 1990; Matson et al., 2012) and make tendons the essential source of elastic storage and recovery during locomotion (Roberts, 2016). The hysteresis values (∼17–18%) measured here were much higher in comparison to the *in vitro* studies mentioned above. Aside from differences due to methodology, such a high hysteresis may indicate a lower energy-conserving capacity and a greater heat accumulation during prolonged exercise. Nonetheless, the findings are in accordance with previous *in vivo* measurements (Finni et al., 2013 for review). Regardless of the magnitudes measured here, the results of this study suggest that tendinopathy does not affect the hysteresis and thus the performance of the myotendinous complex during locomotion or risk of further injury via intra-tendinous hyperthermia. However, this finding contrasts with observations of higher hysteresis, lower storage and release of elastic energy and performance deficits in elite

athletes with mid-portion Achilles tendinopathy (Wang et al., 2012) and could suggest that tendinopathy affects the mechanical properties of patellar and Achilles tendons differently. Such tendon-specific discrepancies are in line with the distinct functional (Kurokawa et al., 2001), metabolic (Kubo and Ikebukuro, 2012), and anatomical (Basso et al., 2001; Szaro et al., 2009) features reported previously. Moreover, these observations highlight that advanced stage tendinopathy may affect the various mechanisms driving tensile loading and unloading of the patellar tendon differently.

The significant correlations found between VISA-P scores and the differences in proximal CSA, stiffness and Young's modulus (**Figure 4**) of affected individuals and healthy controls reinforce the differences found in these variables and suggest that the clinical severity is reflected in tendon properties. The discrepancy between studies measuring the mechanical properties of symptomatic tendons could, therefore, be partly explained by differences in symptom severity as measured with this scale. However, the lack of association between VISA-P and the differences in other objective measures of tendon properties (e.g., capacity to store and release elastic energy) indicates the complexity of this multifactorial pathology. Thus, symptoms typically associated with tendinopathy are not necessarily linked to patient perception nor to mechanical changes in the tendon. The present data do not support the influence of other outcome measures such as the influence of sex, loading pattern, or the occurrence of clinical signs such as neovascularization and/or calcification on tendon properties. These correlation analyses are, however, based on case-control pairs and should be considered with caution. Additional studies with larger cohorts and/or a within-subject design are needed to confirm the observed associations.

Limitations

Potential limitations should be considered before concluding. First, some of the subjects suffered from patellar tendon disorders substantially longer than others (**Table 1**). Although symptom duration reportedly affects tissue cells and extracellular matrix differently (Dakin et al., 2015) a symptom duration difference within the present range of 10–120 months is unlikely to have influenced our results. This assumption is supported by the findings of the present study, with a lack of associations between symptom duration and both function and tendon property differences. Interpretations should, however, be considered in relation to the included cohort with sport-specific high volume (e.g., long-distance running) and high intensity (e.g., volleyball) tissue loading. Second, tensile forces on the patellar tendon are demonstrably influenced by the simultaneous activation of the antagonist's muscles (Maganaris, 2001). However, muscle strength cannot be measured directly *in vivo*, and the quantitative nature of the EMG-strength relationship remains a topic of scientific debate. Despite these potential validation limits, it is unlikely that differences in coactivation have influenced our findings. Similar ratios of antagonist-to-measured knee extension torque in tendinopathic (11.2%) and healthy subjects (10.1%) support this assumption. Finally, tendon provides viscoelastic behavior, and tissue property acquisitions potentially differ during dynamic, real-life situations (Kösters et al., 2014). However, the methodology used in the present study is currently the only way to estimate tendon properties validly and reliably *in vivo*. Moreover, it seems a fair assumption that tendon properties calculated during isometric ramp contractions would be related to their measurements under different contractile conditions. Therefore, this standardized procedure seems valid and does not mitigate our initial conclusions.

CONCLUSION

The comparison of case-control pairs of symptomatic and pain-free, visually asymptomatic patellar tendons confirmed morphological differences and indicated differences in certain mechanical and material properties. Tendinophatic patellar tendons were characterized by a much larger thickness but a lower stiffness (and consequently a lower Young's modulus) at maximal individual force. On the other hand, analyses of the loading and unloading curves suggest that elastic energy storage and dissipation capacities remain similar in tendinopathic and healthy subjects. Besides, the significant correlations found between the functional severity of the pathology (VISA-P) and mechanical and material patellar tendon properties (**Figure 4**) calls for caution when interpreting findings on a group mean level only. The severity of the pathology also affects patellar tendon mechanical properties. Future studies are needed to evaluate if these differences in tendon properties can be used as a predictor of pathology occurrence and whether they are reversible. In addition, upcoming studies should also assess the possible impact of the lower patellar tendon stiffness on muscular function in sporting or daily activities.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the German register for clinical trials: DRKS00011338 Local Research Ethics Committee: 415-E/2012/11-2016. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

H-PW, OS, AK, EM, and FR contributed to the conceptualization of the study, and the review and editing of the writing. H-PW contributed to the methodology, the writing – original draft

visualization, and the supervision. H-PW and FR contributed to the investigation and the project administration. H-PW contributed to the data analysis. FR, H-PW, and OS contributed to the funding acquisition. All authors read and approved the content of the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00704/full#supplementary-material

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Individualized Muscle-Tendon Assessment and Training

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The interaction of muscle and tendon is of major importance for movement performance and a balanced development of muscle strength and tendon stiffness could protect athletes from overuse injury. However, muscle and tendon do not necessarily adapt in a uniform manner during a training process. The development of a diagnostic routine to assess both the strength capacity of muscle and the mechanical properties of tendons would enable the detection of muscle-tendon imbalances, indicate if the training should target muscle strength or tendon stiffness development and allow for the precise prescription of training loads to optimize tendon adaptation. This perspective article discusses a framework of individualized muscle-tendon assessment and training and outlines a methodological approach for the patellar tendon.

Keywords: diagnostic, ultrasound, mechanical properties, performance, individualization

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INTRODUCTION

It has been long recognized that the functional properties of muscles are a crucial determinant of movement performance in both every day and athletic activities (Masterson, 1976; Delecluse, 1997). Therefore, their assessment, especially in terms of muscle strength, is now a standard diagnostic component when monitoring for example performance in sports (Smith et al., 2002; McMaster et al., 2014) or the recovery process in rehabilitation (Osternig, 1986). In comparison, we just recently began to understand how tendons influence muscle-tendon unit (MTU) functioning and performance (Kawakami and Fukunaga, 2006; Roberts, 2016). In the practical field of sports and rehabilitation, the assessment of tendon properties is until now mostly confined to medical imaging in the context of injuries (Robinson, 2009). In this article, we want to make an argument that a differentiated diagnostic of muscle functional and tendon mechanical properties could be a promising approach to individualize training loads. The approach would allow to specifically target muscle or tendon adaptation and facilitate a balanced development of the contractile and series elastic elements of the MTU. Developing effective strategies how to manipulate the interaction of muscle and tendon could make an important contribution for the development of physical performance as well as the prevention and rehabilitation of injuries.

Owing to systematic research endeavors of this century, it is now clearly established that human tendons can adapt to mechanical loading across the lifespan (Waugh et al., 2014; Bohm et al., 2015; McCrum et al., 2018). However, there is also evidence that the functional properties of muscles and the mechanical properties of tendons do not necessarily change in a similar manner during a training process (Mersmann et al., 2017a). For example, tendons do not adapt as quickly to

mechanical loading as muscles due to a lower rate of tissue renewal (Heinemeier et al., 2013). Further, not all types of loading that increase muscle strength are effective in stimulating an increase of tendon stiffness, which is the resilience of the tendon according to its force-elongation relationship. For example, plyometric training and fatiguing training with moderate loads show clear effects on muscle strength and hypertrophy (Sáez-Sáez de Villarreal et al., 2010; Schoenfeld, 2013), yet lower, less consistent or even no effects on the stiffness of the tendon (Arampatzis et al., 2007a; Burgess et al., 2007; Kubo et al., 2007; Bohm et al., 2014). If an increase in the muscle's capacity to generate force is not accompanied by an adequate increase in tendon stiffness, the tendon is subjected to higher levels of strain during a muscle contraction at a given relative intensity. As the ultimate strain of tendons is remarkably constant (LaCroix et al., 2013), an increase of tendon operating strain during muscle contraction implies an increase of the mechanical demand placed upon the tendon.

An imbalanced development of muscle and tendon has implications for (a) movement performance, (b) the risk of injury and (c) the prescription of training loads. Though movement performance is certainly a complex interplay of musculoskeletal (Cormie et al., 2011; Suchomel et al., 2016), neural (Yarrow et al., 2009) and psychological factors (Raglin, 2001), the interaction of muscle and tendon is an integral part with regard to how we produce forces to move. Although on an individual level there is little information concerning muscle-tendon imbalances and specific competitive performance, there are reports that for optimal muscle interaction, muscle strength and tendon stiffness need to be well matched (Lichtwark and Wilson, 2007; Orselli et al., 2017) and controlled via a finely tuned neural drive to the muscle (Sawicki et al., 2015). An imbalance in muscle and tendon adaptation might impair this interplay, which would reduce the efficiency of the musculotendinous energy exchange. Moreover, an increase in operating strain reduces the tendon safety factor (ratio of operating strain to ultimate strain) and may increase the risk of injury. The initial strain induced in a tendon at a given load determines the time to rupture during both static and cyclic loading (Wren et al., 2003). That is why strain-induced tissue damage is considered one of the major mechanical risk factors for the development of tendinopathy (Fredberg and Stengaard-Pedersen, 2008; Lavagnino et al., 2008; Wang et al., 2013). Finally, potential imbalances in muscle and tendon adaptation imply that the prescription of training loads for the tendon is not precise when it is based on the strength capacity of the muscle (e.g., setting the training intensity to a percentage of the one-repetition- or isometric maximum). An effective training stimulus for the tendon is expected at contraction-induced tendon strains of 4.5 to 6.5% (Arampatzis et al., 2007a, 2010; Bohm et al., 2014), which does not correspond to the same intensity of muscle contraction for each individual. Therefore, a differentiated diagnostic of muscle and tendon properties would open up opportunities to optimize loading during training and, thus, facilitate adaptation for the improvement of physical performance or the prevention and rehabilitation of overuse injuries.

FRAMEWORK OF THE INDIVIDUALIZED MUSCLE-TENDON ASSESSMENT AND TRAINING

Tendons, as mostly collagenous structures, are not able to contribute to the active force generation of the muscle-tendon unit. However, due to their compliance, they can significantly affect muscle force production and, thus, are an important component of the human musculoskeletal system for effective locomotion (Roberts, 1997; Lai et al., 2014). Several studies in the last 10-15 years provided important information regarding the Achilles tendon and aponeurosis deformation during different tasks as for example for walking (Lichtwark et al., 2007; Lai et al., 2015), running (Lichtwark et al., 2007; Albracht and Arampatzis, 2013; Lai et al., 2018) and jumping (Kurokawa et al., 2003; Lichtwark and Wilson, 2005; Ishikawa and Komi, 2008). The reported maximum strains of the Achilles tendon during these activities were calculated from muscle fascicle behaviour and range between 4.3% during walking (Lichtwark et al., 2007) up to 9.0% strain in fast running (Lai et al., 2018). Furthermore, current studies investigating the function of the knee extensor MTU evidenced significant deformation of the quadriceps and patellar tendon during jumping (Nikolaidou et al., 2017), landing (Hollville et al., 2019), walking and running (Bohm et al., 2018). These findings demonstrate that a certain deformation of tendons is required during daily life and sport activities for an effective locomotion. This tendon deformation is important because it affects both the force-length-velocity and power-velocity potential of the muscle (Nikolaidou et al., 2017; Bohm et al., 2018, 2019) as well as strain energy storage and return within the MTU (Lichtwark and Wilson, 2005; Ishikawa and Komi, 2008; Lai et al., 2014). Consequently, the muscle has to be strong enough to appropriately deform the tendon and to use tendon elasticity for an efficient muscle-tendon interaction during movement. However, both too high and too low levels of habitual deformation may be associated with impairments of tendon structure. Though the exact ultimate strain of human tendons cannot be determined *in vivo*, it is clear that excessive tendon deformations increase the mechanical demand for the tendon, since *in vitro* data shows that ultimate tendon strain is remarkably constant (LaCroix et al., 2013). Therefore, high operating to ultimate strain ratios increase the risk of tissue failure (Wren et al., 2003). Wang et al. (2013) demonstrated that cyclic strains of 9.0% act degenerative on the tendon structure and weaken its structural integrity. However, the study also provided evidence that also too low deformations ($\leq$ 3.0% strain) may induce catabolic signaling and matrix deterioration.

In a series of systematic intervention studies, we modulated tendon strain magnitude (3% and 4.5–6.5%), frequency (0.17 and 0.5 Hz), strain rate (modulated via time to peak force of $\sim$130 and $\sim$380 ms) and duration (1 s, 3 s and 12 s) while controlling for overall loading volume. We found that cyclic loading of the tendon with strain values between 4.5 and 6.5% and a duration of

3 s per repetition (applied with the low frequency and strain rate) was the most effective mechanical stimulus for the improvement of human tendon mechanical properties *in vivo* (Arampatzis et al., 2007a, 2010; Bohm et al., 2014). Tendon exercise loading with strain values of ∼3.0% on the other hand did not improve tendon mechanical properties (Arampatzis et al., 2007a, 2010). In accordance with our findings, also other authors conclude from their recent experimental results (Wang et al., 2013) or literature review (Pizzolato et al., 2018; Docking and Cook, 2019) that there is an optimal range of tendon strain during exercise for triggering tendon adaptation and promoting its mechanical and morphological properties. The deformation of the tendon during exercise can be regulated by the muscle force generation and strains of 4.5 to 6.5% are usually achieved at about 90% of a voluntary maximum isometric contraction (MVC; Arampatzis et al., 2007a, 2010; Bohm et al., 2014). However, on the individual basis, this might not necessarily be the case.

The maximum muscle strength and tendon stiffness are the two parameters that regulate maximum strain of the tendon during muscle contractions. An imbalance between muscle strength and tendon stiffness can result in either too low or too high tendon strain during maximum contractions with potential negative consequences for both performance capabilities and tendon health (Mersmann et al., 2017a, 2019). In general, there is a strong association between muscle strength and tendon stiffness, at least in triceps surae and quadriceps MTUs. This has been reported for children (Waugh et al., 2012; Pentidis et al., 2019), adolescents (Charcharis et al., 2019; Mersmann et al., 2019), young (Arampatzis et al., 2007b; Epro et al., 2019) and old adults (Stenroth et al., 2012; Epro et al., 2017). **Figure 1** shows the correlation of plantar flexor muscle strength with Achilles tendon stiffness and quadriceps muscle strength with patellar tendon stiffness in 172 and 215 athletes, respectively. The significant association between muscle strength and tendon stiffness in both MTUs support the idea that, in general, muscle strength and tendon stiffness show a coordinated adaptation and that individuals with higher muscle strength also have stiffer tendons. However, a significant relationship between muscle strength and tendon stiffness does not give evidence to a balanced adaptation within the MTU, because a high or low relationship does not provide any information concerning the margin of tolerated mechanical tendon loading during MVCs. There is experimental evidence of imbalances between muscle strength and tendon stiffness in competitive athletes from child- to adulthood due to different alterations of muscle and tendon properties, resulting in remarkably high or low tendon strain values (Mersmann et al., 2016; Charcharis et al., 2019; Pentidis et al., 2019). Those imbalances indicate the relevance of an individualized training control and regulation. If the maximum tendon strain during an MVC is too high (>9.0%), tendon stiffness seems too low compared to the strength capacity of the associated muscle and we would recommend a training that focusses on tendon adaptation (i.e., loading that causes 4.5 to 6.5% tendon strain in five sets of four repetitions with a loading-unloading duration of 3 s each and an inter-set rest of 2 min according to our recommendations; Mersmann et al., 2017a). If, on the other hand, the maximum strain is quite low (<4.5%), muscle strength seems too low compared to the stiffness of its tendon and a training that focused on muscle growth is indicated. Such scenarios can occur on an individual basis in athletes and need an individualized training regulation within the MTU. The strain levels suggested are not to be understood as cut-off criteria for injury prediction or fixed thresholds yet as transition bands into high or low levels of maximum strain. This information then can be used to individualize training, aiming to counteract muscle-tendon imbalances. In the authors' view and considering the experimental data reviewed here, it is likely beneficial for performance capacity and injury risk if tendon stiffness is geared to muscle strength.

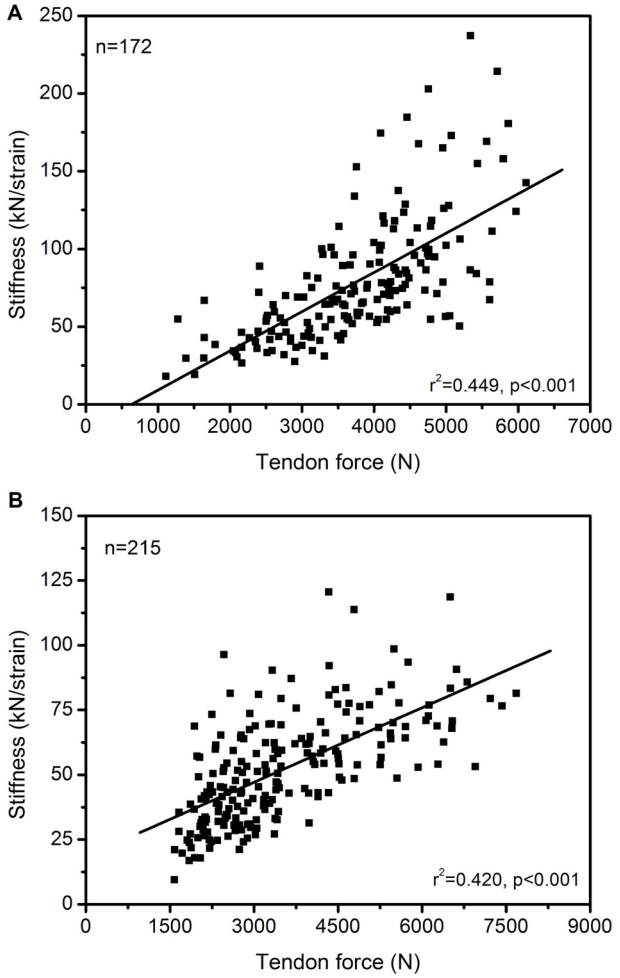

FIGURE 1 | Association between *in vivo* Achilles **(A)** and patellar **(B)** tendon force and tendon stiffness (normalized to tendon rest length) in 172 and 215 athletes from different sports (endurance running, sprinting, ball sports, diverse) and untrained individuals, including data from adolescents and adults. The Pearson correlation coefficients and 95% confidence intervals [lower limit, upper limit] were 0.670 [0.578, 0.745] and 0.648 [0.563, 0.719] for the Achilles and patellar tendon, respectively. The presented data is from earlier studies of our group (A: Arampatzis et al., 2007a,b, 2010; Stafilidis and Arampatzis, 2007; Albracht and Arampatzis, 2013; B: Charcharis et al., 2019; Mersmann et al., 2019) as well as yet unpublished work (A: n = 26; B: n = 118).

In our opinion, the assessment of the appropriate relationship between maximum muscle strength and tendon stiffness, using the maximum tendon strain during an MVC as diagnostic marker, is important for the training process. Imbalances between muscle strength and tendon stiffness can be identified in an early stage and customized decisions can be made for the training regulation of the individual athlete. **Figures 2A,B** shows the maximum strain values of the Achilles and patellar tendon of a high number of athletes during an MVC. It is visible that there are athletes who show either markedly high or low strain values and, thus, we would suggest personalized justification with focusing on muscle strength or tendon stiffness training, respectively. In athletes with maximum tendon strain higher than 11.0% the specific training to increase tendon stiffness seems crucial, while in others with strain values of 9.0 to 10.0% a slight correction in training content might suffice. There are also athletes that show maximum strain values <4.5%, which suggests that a customized training for muscle hypertrophy to increase muscle strength might be beneficial.

The relationship between muscle strength and tendon stiffness is further important for the definition of the optimal exercise intensity for tendon adaptation. It is well accepted that both muscle hypertrophy as well as muscle strength can be improved using low intensity exercise (e.g., 30% of one-repetition maximum) with high number of repetitions until fatigue (Mitchell et al., 2012). However, low intensity exercise does usually not initiate sufficient tendon strain to initiate adaptive changes of tendon properties (Bohm et al., 2015; Wiesinger et al., 2015). As mentioned above, the effective mechanical loading for tendon adaptation should cause tendon strains between 4.5 and 6.5%, which corresponds in average to a tendon loading of 90% MVC (Arampatzis et al., 2007a, 2010; Bohm et al., 2014). The individual and different relationship between muscle strength and tendon stiffness in athletes implies, however, that there can be substantial variations in terms of the percentage of the MVC at which the target levels of tendon strain for training are reached (**Figure 2C**). Therefore, the individual assessment of the MVC-strain relationship of the tendon is relevant for the definition of the optimal loading intensity, since it allows to individually fit the target strain (4.5–6.5%) to the MVC for a personalized tendon training.

It has to be mentioned that the origin of tendon pathology is multifactorial and currently there is not a clear factor or concert of factors that explain or precisely predict the occurrence of tendinopathy (Magnusson et al., 2010; Cook et al., 2016). The prevention of imbalances within the muscle-tendon unit might, however, reduce the risk of overload and, thus, a key risk factor of tendinopathy, while other risk factors related to genetics, age or recovery time and others certainly still contribute to tendon collagen turnover, pathology and function. Our proposed approach is intended to be used in the practical field to detect at an early stage if a tendon is in an unfavorable loading environment due to muscle-tendon imbalances. It is then possible to prescribe individualized training recommendations aiming to promote an efficient energy exchange between muscle and tendon and to counteract the potential development of overuse. Several additional methodologies including ultrasound

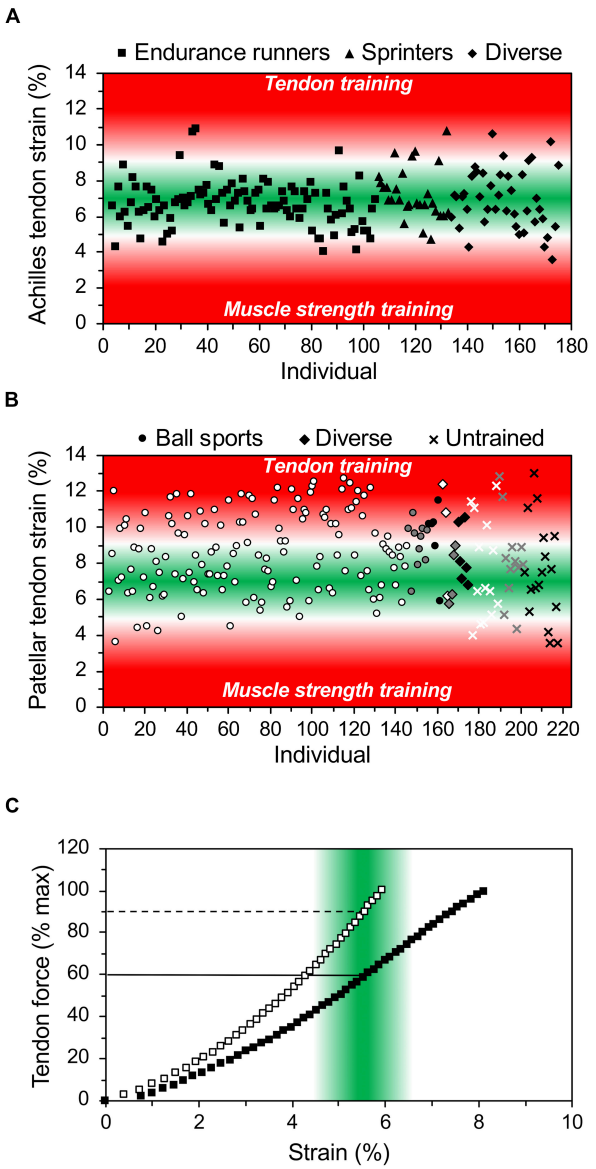

FIGURE 2 | *In vivo* Achilles **(A)** and patellar **(B)** tendon strain during maximum voluntary isometric contractions in 172 and 215 athletes from different sports (endurance running, sprinting, ball sports, diverse) and untrained individuals from child- to adulthood (white: early adolescent [12–15 years], gray: late-adolescent [16–19 years], black: adults [≥20 years]). While low levels of tendon strain suggest that the athlete may focus on muscle strength development, high levels of strain indicate the need for specific tendon training for increasing its stiffness. **(C)** Illustrates the individual relationship of tendon force (in percent of maximum tendon force) and strain in two athletes. The green area indicates the range of strain where an optimal mechanical stimulation for training is expected and the horizontal lines show that the respective relative training intensity in terms of force exertion may differ substantially between individuals. The presented data is from earlier studies of our group (A: Arampatzis et al., 2007a,b, 2010; Stafilidis and Arampatzis, 2007; Albracht and Arampatzis, 2013; B: Charcharis et al., 2019; Mersmann et al., 2019) as well as yet unpublished work (A: n = 26; B: n = 118). Strain was extrapolated based on stiffness for the tendon forces during maximum voluntary isometric contractions in the respective optimum joint angle. For details on the respective methods see Arampatzis et al. (2007a) and Mersmann et al. (2016).

tissue characterization (van Schie et al., 2010; Visnes et al., 2015), spatial frequency analysis of ultrasound images (Bashford et al., 2008; Mersmann et al., 2019), intra-tendinous motion (Couppé et al., 2020), tissue biopsy and microdialysis (Magnusson et al., 2010) can be used to improve our understanding of tendon pathogenesis and function.

POTENTIAL PRACTICAL IMPLEMENTATION AT THE EXAMPLE OF THE PATELLAR TENDON

Individualizing exercise prescriptions for muscle and tendon training requires an assessment of muscle strength and tendon mechanical properties. The measurement of a tendon force-elongation relationship *in vivo* is, however, associated with considerable methodological effort (Seynnes et al., 2015). But, allowing for some simplified assumptions, it seems possible to develop a diagnostic setup for the application in the field. First, as tendon force is approximately proportional to the generated joint moment during isometric contractions, the assessment of the tendon moment arm and calculation of tendon forces could be omitted. The relationship of externally measured moments or forces to the elongation of the tendon would therefore be representative of tendon stiffness. While interindividual comparisons of such a measure of tendon stiffness would be biased by differences in the tendon moment arm, longitudinal changes should be well represented as long as no major change of moment arm within individuals can be expected (i.e., in adults). Second, though there might be differences in antagonist coactivation between untrained and trained cohorts that affect the ratio of externally measured force or moment to the actual tendon force, after a few accustoming sessions no major changes in the relative contribution of the antagonist to the resultant joint moment are to be expected (Carolan and Cafarelli, 1992). Recently, we measured knee joint moments in 14 adolescent basketball athletes at four measurement time points of a competitive season and observed only marginal fluctuations of the antagonist moment of 2.5 $\pm$ 1.5%. Third, while the elaborate assessment of tendon cross-sectional area is necessary to understand the mechanisms of tendon adaptation in the scientific field, for monitoring training adaptations and prescribing exercise it seems sufficient to confine the outcome parameters to tendon stiffness or even only to tendon strain.

Tendon mechanical properties *in vivo* are measured during isometric contractions. For the assessment at the patellar tendon, we would suggest a seated position with the knee flexed to 90°, as in this position passive forces resolve tendon slack (yet not causing substantial elongation), which simplifies the measurement of tendon rest length and elongation, and the alignment of the force sensor with the force vector can be more easily controlled. It needs to be mentioned that a 90° knee joint angle not optimal for maximal moment generation and can result in lower tendon strain values, which needs to be kept in mind in their interpretation. After a standardized warm-up and a series of at least 5 submaximal isometric contractions as preconditioning for the tendon (Maganaris, 2003), the participant performs isometric ramped contractions with a gradual increase in force exertion from rest to maximum in about 5 seconds. The elongation of the patellar tendon during the contractions is visualized time-synchronized with the force or moment data using a linear ultrasound transducer overlying the tendon in the sagittal plane aligned with its longitudinal axis. Though the availability is currently limited, the use of a long linear ultrasound transducer (>6 cm) is to be recommended as it enables the visualization of the tendon origin and insertion at the patella apex and tibial tuberosity in one image (Mersmann et al., 2018). The displacement of the tendon insertion is currently tracked using self-developed manual tracking interfaces (e.g., Mersmann et al., 2017b) or (semi-)professional video analysis software (e.g., Image J®, Tracker®). Fully automated tracking might in near future replace these time-consuming procedures and enhance the objectivity of the analysis. To achieve a high reliability of the elongation measurement, three to five trials should be recorded and averaged (Schulze et al., 2012). The slope of a linear regression of the external force (or moment) and tendon elongation data between 50% and 100% of the exerted maximum force would be calculated as representative of tendon stiffness.

Due to its crucial importance for the estimation of injury risk, efficient muscle-tendon interaction and, thus, exercise prescription, an even more simplified assessment of tendon properties could be confined to tendon strain as outcome parameter. In such an approach, it would not be necessary to track the tendon insertion points over the full course of the contraction, yet only at rest and the plateau of the isometric maximum. In that case, the synchronization of ultrasound and force or moment data could be spared as well and tendon rest length and maximum elongation could theoretically be measured using the built-in software of the ultrasound device. Certainly, a validation and assessment of the reliability of the proposed procedures would be necessary and details still needs to be established how the approach can be most sensibly applied in different sports and environments in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

Ethics statements considering previously published work of our group can be found in the respective publications. Regarding the yet unpublished data, the participants (and legal guardians where applicable) gave written informed consent to the experimental procedures, which were approved by the Ethics Committee of the Humboldt-Universität zu Berlin (Ethikkommission der Kultur-, Sozial-, und Bildungswissenschaftlichen Fakultät; 16.02.2018) or the Ethics Committee of the Charité (Ethikausschuss 2 am

Campus Virchow-Klinikum; ref. nr. EA2/076/15) and carried out in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

AA, FM, and SB conceived the presented approach and drafted the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for the content of the work.

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PERSPECTIVE
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A Perspective on Reversibility of Tendinosis-Induced Multi-Level Adaptations

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Achilles tendinopathy is a well-known pathology that can display interindividual variations in chronicity, symptom presentation, and tendon morphology. Furthermore, symptoms may fluctuate within an individual throughout the stages of the pathology. Although pain is often used as a marker of condition severity, individuals may not consistently report pain due to periods of remission. Persons with tendinosis, which is characterized by advanced morphological alterations, have shown consistent changes in neuromechanics that indicate adaptations in the sensory-motor and the central nervous systems. The current treatment strategy involves repetitive resistance exercise aiming to achieve recovery of lost function. This treatment approach, however, has gauged such functional recovery through symptom relief and return to sport, which, in our opinion, may not suffice and may not prevent symptom recurrence or tendon rupture. In this physiologically informed perspective, we briefly review what is currently known about the consequences of Achilles tendon degeneration and examine the topic of reversing these changes. Shortcomings of contemporary treatment strategies are discussed and we therefore call for a new paradigm to focus on the whole-body level, targeting not only the tendon but also the reversal of the neuromotor control system adaptations.

Keywords: tendinopathy, motor control, stiffness, feedforward, neuromechanics, spinal reflex, plasticity

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INTRODUCTION

Achilles tendinosis, a subcategory of Achilles tendinopathy characterized by advanced degeneration, continues to lack interventions capable of consistently reversing this degeneration and its multi-system consequences, despite the significant attention garnered in the literature. The absence of consensus on a term for capturing the combination of advanced degeneration and potentially intermittent pain (Cook et al., 2016; Scott et al., 2019) has led us to adopt the use of tendinosis, which we operationally define as imaging-quantified, increased focal tendon thickness and intratendinous disorganization with past or current tendon pain.

Our framework espouses the earlier views that the tendinopathic process begins with paratenonitis and may lead to tendinosis (Hess et al., 1989; Kannus and Józsa, 1991). Marked changes due to experimentally-induced, short-term tendinosis in rabbits were more frequent in the paratenon than in the tendon proper (Backman et al., 1990). In addition, such marked tendon changes rarely occurred without concurrent alterations in the paratenon. Similarly, in humans, a greater proportion of paratenon abnormalities in Achilles tendinopathy were accompanied by intratendinous changes as compared to intratendinous abnormalities showing

concomitant paratenon changes (Karjalainen et al., 2000). This pattern continues to hold true in the case of ruptured Achilles tendons in humans: early histopathological evidence included degeneration in the tendon proper with no signs of inflammatory infiltrates (Kannus and Józsa, 1991). With regards to connections between the various processes, recent findings that inflammatory mediators seemed to stimulate the preliminary changes of tendinopathy further imply that the processes of inflammation and degeneration are not discontinuous (Behzad et al., 2013). As such, we view Achilles tendinopathy as a continuum in which the initial stage is inflammation-and-pain-dominated paratenonitis while the degenerative tendinosis stage occurs if the offending stimulus is not attenuated.

It is important to note that pain appears to follow a stimulus-response relationship and is not always reported, complicating the detection of degeneration (Maffulli et al., 1998, 2003). This is exemplified by instances where imaging of the Achilles upon rupture or first indication of pain has revealed advanced degeneration, indicating the degeneration must have begun in the absence of pain (Maffulli et al., 2003; Cook and Purdam, 2008). However, current treatment strategies often focus on pain, which can be improved in as little as 2 weeks (Murphy et al., 2018), and do not integrate recently discovered multi-level adaptations that occur in the wake of degeneration (Chang and Kulig, 2015). Therefore, there is a need to determine whether these treatment strategies are capable of reversing Achilles degeneration and its associated sequelae.

The aim of this perspective is to examine the topics of Achilles tendon degeneration, reversal of said degeneration, and the determination of whether a new treatment approach for Achilles tendinosis is warranted. From the whole-body perspective, we speculate that pain may leave a footprint on movement strategies, especially those of a repetitive nature, such as running, jumping, or cutting. Only during the pain-free period can the participant tolerate loads that stimulate material and mechanical property enhancement, giving the tendon the chance to remodel. A comprehensive treatment protocol will address not only the tendon tissue properties, but will also modify movement strategies. This statement comes with the qualifiers that (1) improvement of tendon tissue properties must precede the movement strategy modifications and (2) each portion of the treatment plan avoids inducing levels of pain that may interfere with the desired mechanical loading.

MORPHOLOGICAL CONSIDERATIONS IN ACHILLES TENDINOSIS

The degenerative changes of the Achilles tendon occur on both the intratendinous (micromorphological) and the whole-tendon (macromorphological) scales, with the latter category typically referring to non-uniform changes in tendon thickness and cross-sectional area (Cook and Purdam, 2008; Kulig et al., 2013, 2014). In contrast, micromorphological alterations are much more extensive but are not always detected due to a lack of commonplace assessment outside the research setting. Sonography (with macro and micromorphology analysis) is our recommended modality as it allows for ease of access in the clinical setting and also permits analysis of the intratendinous structural integrity using recent techniques (Bashford et al., 2008; Van Schie et al., 2010).

The numerous micromorphological changes of tendinosis include decreased collagen density; disruption of collagen orientation and organization; increased sonographic hypoechogenicity; angiogenesis; and possible ingrowth of nerves (Riley, 2004; Cook and Purdam, 2008; Xu and Murrell, 2008; Klatte-Schulz et al., 2018). Although the tendinotic Achilles may show these changes at the myotendinous junction or insertion, they are most common in its mid-substance, where tendon thickening is localized (focal thickening). Ultrasound-based analyses indicated that increased focal thickness in the presence of degeneration, unlike homogenous thickness, involves separation of collagen bundles (Bashford et al., 2008; Kulig et al., 2016), which was associated with alteration of tendon material and mechanical properties. This increased distance between axially aligned collagen bundles within a given region of focal thickness was recently correlated with lower tendon modulus of elasticity and stiffness (increased compliance) (Kulig et al., 2016), providing evidence for the morphological underpinnings of tendon material and mechanical properties.

MULTI-LEVEL PHYSIOLOGICAL ADAPTATIONS TO TENDINOSIS

The functional consequences of long-term Achilles tendinosis extend outside the tendon to the muscle and nervous systems (Arya and Kulig, 2010; Chang and Kulig, 2015). The following adaptations were revealed through studies from our lab on subjects who had previously experienced various levels of Achilles tendon symptoms but were pain-free at the time of participation in our studies. At the tendon level, degeneration affects material and mechanical characteristics. Reductions in these properties then lower the tendon's resistance to strain and elongation. One manifestation of this tendon compliance is increased electromechanical delay (EMD) of the triceps surae, representing the lag between muscle activation and measurable force production (Chang and Kulig, 2015). The nervous system then attempts to compensate for this temporal inefficiency by upregulating the feedforward drive, observed as earlier pre-activation of the gastrocnemius prior to the point of initial ground contact during hopping.

In addition to the supraspinal involvement implied by the aforementioned feedforward signal, adaptations to advanced tendon degeneration are also observed at the spinal level (Chang and Kulig, 2015) in the form of feedback control mechanisms. Tibial nerve motor neurons supplying the triceps surae have displayed a higher normalized H-reflex, indicating increased alpha motor neuron excitability. The altered excitability may result in part from enhanced descending neural drive from the cortex, seen as an increase in the V-wave from the tibial nerve. The presently known adaptations conclude with depression of triceps surae and tibialis anterior electromyography (EMG) amplitude. Although this reduced recruitment of the primary

plantar flexors and their antagonist may occur to shield the tendon from further load, adequate force production then demands use of the secondary plantar flexors. As a result, there is increased peroneus longus activity relative to the gastrocnemius and soleus. It is reasonable to speculate that there is also altered recruitment of the other secondary plantar flexors, keeping in mind that this is currently unconfirmed. The myriad of alterations presents the challenge of identifying intervention protocols that rectify not only the tendon's inherent properties, but that address nervous system-based adaptations occurring at the whole-body level.

SIGNIFICANCE OF REVERSIBILITY

The understanding of the multilevel adaptations to Achilles tendinosis can benefit from considering the human motor control perspective. In human motor control terms, the tendon is the bridge between the actuator (muscle) and the plant (i.e., the skeletal system). In the presence of Achilles tendinosis, the tendon is weakened, impacting the actuators, the sensors (proprioceptors), and the controller (nervous system). The temporal efficiency of the musculotendinous unit is compromised, stimulating the central nervous system to tune both feedforward and feedback control mechanisms as compensation for the deficit of motor output. If the tendon regains its normal function, it then becomes possible to reverse the adaptations seen in the actuator and controller. The reversal of the multilevel adaptations is therefore critical in treating persons with Achilles tendinosis, and intervention should start with the enhancement of tendon modulus of elasticity and stiffness.

REVERSIBILITY STRATEGIES

It is our informed hypothesis that the multilevel adaptations accompanying tendinosis (Chang and Kulig, 2015), likely developed over many years, exhibit the potential for reversal. Integrating the intervention-based literature with the documented adaptations can guide strategies aimed toward producing this reversal. The motivation underlying the interventions seen in the current literature originates from two complementary areas; one stems from aging and sports to target enhancement of performance while the second, based in tendinopathies, sets the goal of returning to prior activity after abolishing pain. Both research communities target the local tissue, despite the mechanical nature and demand of the first and the analgesic roots of the other.

It was established as far back as 2010 (Arya and Kulig, 2010; Child et al., 2010) that morphological changes within a tendon, attributed to degeneration, are accompanied by diminished intratendinous mechanical and material properties. These impaired tendon mechanics have given rise to intervention strategies, typically seen as a progressive resistance exercise program in the performance enhancement literature (Bohm et al., 2015). However, pathology carries with it three common issues that do not readily come to mind when the primary goal is limited to performance. The first issue relates to tendon morphology: although training-induced increases in Achilles tendon loading stimulate uniform increases in collagen production as seen by hypertrophy and improved elastic modulus (Bohm et al., 2015; Wiesinger et al., 2015; Magnusson and Kjaer, 2019), the pathological tendon's thickening is non-homogenous (Maffulli et al., 2003; Cook and Purdam, 2008; Magnusson and Kjaer, 2019). The focal thickening is attributed to retention of water (Cook and Purdam, 2008; Magnusson and Kjaer, 2019) and thinner, disorganized collagen fibers (Maffulli et al., 2003), explaining the heightened mechanical compliance. This difference in morphological response begs the consideration that the pathological tendon may not respond to resistance exercise with the same efficiency as the healthy tendon.

The second issue is attributed to the time course of adaptations to tendon degeneration. We expect that the peripheral and central adaptations to a compliant tendon occurred over an extended time interval, and once established, are continuously reinforced by the persistence of said compliance. The late adaptations may require more response time under traditional treatment, reducing the efficiency of such protocols. The final matter is that presence of pain alters the body's ability to undergo and respond to resistance exercise (Henriksen et al., 2011). Despite these challenges, tendon-targeted, slow progressive loading remains a viable strategy to initiate reversal of tendon alterations and the accompanying adaptations.

Previous reports indicate reduction of pain in as little as 2 or 3 weeks, suggesting that marked changes in the discussed alterations and adaptations will require longer periods of time (Murphy et al., 2018). On the other hand, the time-course of change in healthy tendon's stiffness, due to resistance exercise, averages 12–14 weeks (Arampatzis et al., 2007, 2010; Kubo et al., 2010, 2012; Fouré et al., 2013; Wiesinger et al., 2015) with a suggested minimum of 8 weeks for a large effect (Bohm et al., 2015). Improvements in the elastic modulus show a similar trajectory. In contrast, alterations in cross-sectional area seem to require different exercise parameters. A meta-analysis on the effects of exercise on healthy tendons determined that protocols utilizing contractions lasting only one second at loads lower than 80% maximal voluntary contraction (MVC) were unable to induce improvements in cross-sectional area (Bohm et al., 2015). Furthermore, previous interventional studies on Achilles tendinopathy have reported the successful reduction of abnormal cross-sectional area via the use of heavy-resistance training (Magnusson and Kjaer, 2019). Therefore, our overall consensus is to aim for three main components in interventions for Achilles tendinopathy: (1) resistance exceeding body weight, (2) a high number of multi-second repetitions per day (up to 180 repetitions, depending on the stage of intervention), and (3) duration of the program in months as opposed to weeks. We propose that only a sufficient enhancement in tendon stiffness will result in reversibility of long-term neuromechanical adaptations. This statement is purely speculative in nature, largely due to the absence of studies monitoring intervention-based changes in tendon morphological, material, and mechanical properties in combination with the functional effects of these

properties. We would like the readers to note that the above recommendations are merely meant to guide the development of new protocols rather than suggesting specific exercises.

As a means of supporting this proof of concept, we present data from a single subject with unilateral Achilles tendinosis who showed the same neuromechanical adaptations as the participants in Chang and Kulig's 2015 study (Chang and Kulig, 2015). The subject, an active male in his 40's with two prior episodes of tendon pain (10 years and 1 year prior to the data collection, respectively), underwent a progressive-resistance, slow-rate exercise regimen based off of the Alfredson protocol (Alfredson et al., 1998). Prior episodes were managed by active rest. Soon after the onset of the current episode (which included perception of stiffness in the morning and pain during running), the athlete's Achilles tendons were imaged and a significant focal thickness was discovered on the left side. After 2 weeks of active rest, when pain subsided, written informed consent was obtained and laboratory tests were commenced (approved by the IRB at the University of Southern California). Experimental data were collected in a motion analysis laboratory before and after the 12-week exercise regimen (**Table 1**). The parameters of interest are the same as those described in the "Multi-Level Physiological Adaptations" section. For a complete description of the laboratory methodology, please refer to the 2015 manuscript (Chang and Kulig, 2015). Furthermore, to bolster the interpretability of the intervention data, we compared the pre and post data to reliability values from an earlier repeated-measures experiment. The standard error of measure (SEM) values from this latter experiment are available in the 2015 manuscript referenced above.

Taken together, the following information can be gathered from these preliminary data (**Table 1**). The focal thickness of the degenerated tendon diminished by 8% while the tendon stiffness, though still considerably lower than the non-involved side, increased by 24%. There was an observable EMD reduction of 19.5%, followed by delayed onset of gastrocnemius pre-activation (onset is closer to the time of ground contact by 17.3% of the pre-intervention time). In other words, there was improvement in the temporal efficiency of the mechanical system and the body's feedforward control response was subsequently adjusted to match that temporal efficiency. Although these are notable improvements that exceed the minimal detectable change (MDC) for data collected in our laboratory, they did not entirely eradicate the between-limb differences in these parameters. Please see Chang and Kulig (2015) for the standard error of measurement values used to calculate the MDC. Moreover, changes in the reflex and feedback variables as well as task-specific muscle activation patterns did not exceed the MDC.

In **Figure 1A** we present a hypothetical 36-week timeline for the reversal of changes in tendon morphology and associated properties. For clarity, we present the parameters on an arbitrary scale. We acknowledge that the time course of changes will likely be non-linear, with greater response to the stimulus in the early portions of the intervention. However, we suggest that the progressive increase in stimulus, as well as the inherent ability of the degenerated tendon and the organism to continuously adapt, will prevent early cessation of the reversal.

We expect that the first changes will involve the intratendinous morphology. The first levels of external load should induce collagen repair and synthesis, thereby improving the percentage of aligned collagen fibers (Magnusson and Kjaer, 2019). One effect of this improvement in aligned fibers would be to reduce the ratio of proteoglycans to collagen, which should create an impetus for removal of the water in the tendon, ultimately reducing the focal thickness. However, the changes in thickness

TABLE 1 | Morphological and neuromechanical variables before and after a 12-week progressive-resistance heel-lowering exercise intervention.

	Non-tendinotic limb [Mean (SD)]			Tendinotic limb [Mean (SD)]		
	Pre-intervention	**Post-intervention**	**Δ**	**Pre-intervention**	**Post-intervention**	**Δ**
Peak Spatial Frequency Radius† (mm^{-1})	2.12	2.18	0.06	1.63	1.87	0.24
Thickness (mm)	5.13(0.13)	4.83(0.13)	−0.29	8.13(0.06)	7.44(0.11)	−0.69
Stiffness (N/mm)	319.87(19.23)	331.23(21.04)	11.36	182.93(21.08)	227.98(18.30)	45.05
Elastic modulus (MPa)	1375.44(15.7)	1432.05(18.3)	56.61	512.2(13.2)	598.13(14.7)	85.93
EMD§ (ms)	25.67(1.92)	24.91(2.11)	−0.76	39.83(2.17)	32.07(3.13)	−7.76
Pre-activation§ (ms)	65.70(3.74)	66.20(4.51)	0.50	87.30(3.23)	72.20(1.32)	−15.10
H/M ratio‖	0.26(0.11)	0.24(0.09)	−0.01	0.42(0.10)	0.41(0.05)	0.00
V/M ratio‖	0.28(0.19)	0.21(0.11)	−0.07	0.46(0.21)	0.44(0.16)	−0.02
CI#	0.90(0.13)	0.79(0.23)	−0.11	0.45(0.09)	0.55(0.15)	0.09
CCR**	0.53(0.16)	0.58(0.14)	0.05	0.25(0.11)	0.27(0.09)	0.02

*Single subject data are shown in two columns where "tendinotic" refers to the involved limb and "non-tendinotic" refers to the contralateral, non-involved limb. Delta (Δ) represents the difference between the pre- and post-intervention assessments. All data are reported as mean (standard deviation). Standard deviations for each variable were obtained from multiple measurements (3 to 5) on this subject. Shaded numbers indicate Δ exceeded the minimal detectable changes (MDC) for data from our lab. The MDC thresholds were calculated from standard error of measurement (SEM) values derived from reliability tests on five subjects. Please see Chang and Kulig (2015) for the relevant SEMs. †Higher peak spatial frequency radius indicates less separation between collagen bundles (more bundles per mm); §Electromechanical delay (EMD) and pre-activation represent the temporal efficiency of force transmission and the feedforward control response to that temporal efficiency, respectively; ‖H-reflex (H) and V-wave (V) ratios correspond to measures of spinal and supraspinal neural drive, respectively, and are normalized by the maximum M-wave (M) found during peripheral stimulation. #Contribution index (CI) closer to 0 represents increased sagittal-plane synergist activity (peroneus longus) relative to the gastrocnemius and soleus. **Co-contraction ratio (CCR) closer to 0 indicates decreased antagonist activity (tibialis anterior) relative to the three plantar flexors used in the CI variable.*

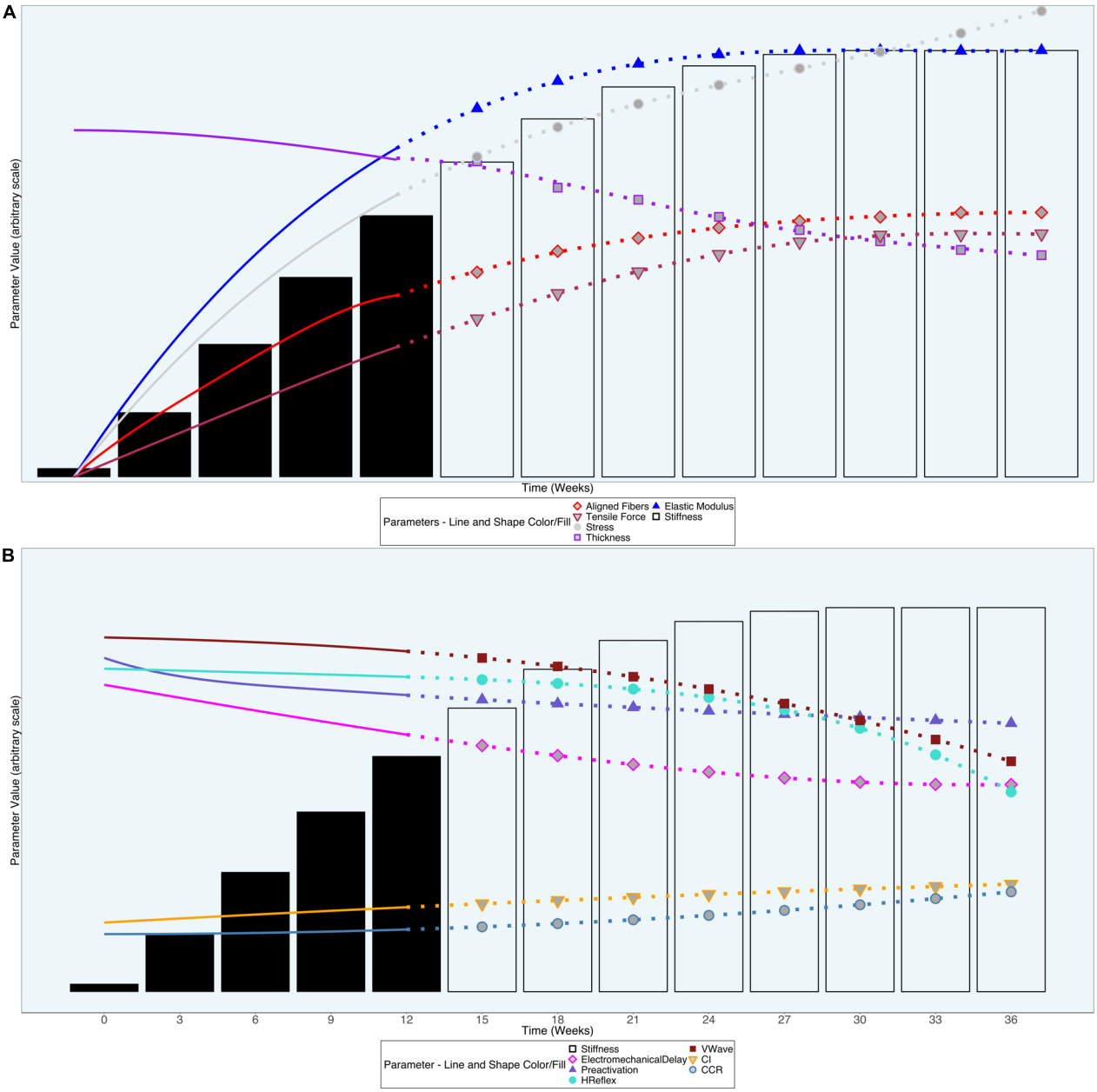

FIGURE 1 | Hypothetical model of the time course of changes that may occur during progressive loading-based training of the degenerated Achilles tendon. **(A)** *Tendon morphological parameters*. Progressive increases in the external load (not depicted) will create concomitant increases in tensile force (red; filled diamonds) and therefore stress (gray; circles). This will in turn stimulate much-needed deposition of collagen but focal thickness reduction will likely outweigh the deposits, reducing the tendon's overall thickness (purple; filled squares) while improving the ratio of aligned to non-aligned fibers (maroon; filled inverse triangles). The overall result is improvement in tendon elastic modulus (blue; triangles) and stiffness (black bars). **(B)** *Neuromechanical adaptations to tendon degeneration*. Once a sufficient improvement in tendon stiffness (black bars) is observed, electromechanical delay (magenta; filled diamonds) will decrease, allowing triceps surae pre-activation onset (purple; triangles) to occur closer to ground contact. Reduced duration of muscle activity may then impact alpha motor neuron excitability (H-reflex; turquoise, circles), descending cortical drive (V-wave; dark red; squares), and the simultaneous activity of multiple muscles (Contribution index and co-contraction ratio; orange and steel blue; filled inverse triangles and filled circles). The unfilled black bars, dotted lines, and symbols in both **(A)** and **(B)** indicate that the corresponding values are hypothetical and require laboratory testing for confirmation. Figure format informed by Wiesinger et al. (2015).

are thought to eventually plateau due to further increases in collagen offsetting the reduction in water content.

The increase in aligned collagen fiber percentage is hypothesized to begin the process of normalizing the modulus of elasticity and stiffness (Wiesinger et al., 2015). We suggest that increases in the modulus of elasticity indicate a greater number of properly aligned and healthy collagen fibers, thus representing a reduction in the ratio of fibers with permanent

versus non-permanent damage. This underlying improvement in healthy collagen fiber numbers would in turn suggest a greater ability of the tendon to resist stress (deforming force normalized by area). On the other hand, fibers that have suffered permanent damage (plastic deformation) would undergo a larger amount of strain (elongation relative to original length) for a given amount of stress. The direct effect of healthy collagen fibers on the modulus of elasticity indicates that improvement in the modulus may occur prior to and may facilitate recovery of tendon stiffness, a notion also suggested by Bohm et al. (2015).

In **Figure 1B**, we present the additional speculative reversibility of supraspinal and spinal adaptations. Once the tendon stiffness increases, the temporal efficiency of force transmission (EMD) should improve, allowing a subsequent delay in the pre-activation onset, thus approximating pre-pathology timing. In theory, the delayed pre-activation would lead the nervous system to decrease the excitability of the alpha motor neuron, thus removing the need for the compensatory heightened H-reflex and V-wave. The co-contraction ratio (CCR) and contribution index (CI) may not de-adapt until there is a sufficient reduction in pain and other symptoms, permitting full utilization of the triceps surae and the opposing dorsiflexors. However, due to the failure of these last four parameters to reach MDC in our lone participant, we currently lack sufficient information to delineate the order and timing of their normalization.

ELIMINATING THE ROOT CAUSE – WHERE DO WE START?

These data from a single subject illustrate that reversing the multi-level adaptations in the motor control system is a reachable goal. However, the current treatment strategy focusing on pain reduction and returning to activity appears insufficient. This is largely a result of the lengthy duration, which would require numerous clinical visits as well as extensive time commitment at home, both of which may affect adherence. To achieve reversal of multi-level adaptations accompanying Achilles tendinosis, the initial stages of rehabilitation should target the morphology and associated mechanical (structural) and material deficiencies of the tendon. The adaptations remaining after this initial stage of mechanical recovery are closely aligned with variables seen in the motor control and motor learning field. This indicates that motor learning strategies aiming to undo adaptation at the higher levels of the control system are good candidates for incorporation into the treatment regimen. Therefore, we conclude this perspective with the assertion that the combination of progressive resistance exercise, motor learning, and currently unknown exercises should prove suitable for reversing the effects of Achilles tendon degeneration and improving the quality of life of those affected.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Southern California, IRB. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KK and Y-JC conceived and designed the study. Y-JC acquired and analyzed the data. KK and Y-JC interpreted the data. KK, Y-JC, and DO-W drafted, revised, and approved the final version of the manuscript.

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Muscle Fascicles Exhibit Limited Passive Elongation Throughout the Rehabilitation of Achilles Tendon Rupture After Percutaneous Repair

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Achilles tendon rupture (ATR) results in long-term functional and structural deficits, characterized by reduced ankle mobility and plantarflexor muscle atrophy. However, it remains unclear how such functional impairments develop after surgical repair. While it is known that this injury negatively affects the tendon's function, to date, limited work has focused on the short-term effect of ATR on the structure of the muscles in series. The aim of this study was to characterize changes in medial gastrocnemius architecture and its response to passive lengthening during the post-surgical rehabilitative period following ATR. Both injured and contralateral limbs from 10 subjects (1 female, BMI: 27.2 ± 3.9 kg/m^2; age: 46 ± 10 years) with acute, unilateral ATR were assessed at 8, 12, and 16 weeks after percutaneous surgical repair. To characterize the component tissues of the muscle-tendon unit, resting medial gastrocnemius muscle thickness, fascicle length, and pennation angle were determined from ultrasound images with the ankle in both maximal plantarflexion and dorsiflexion. The ankle range of motion (ROM) was determined using motion capture; combined ultrasound and motion capture determined the relative displacement of the musculotendinous junction (MTJ) of the AT with the medial gastrocnemius. The ATR-injured gastrocnemius muscle consistently exhibited lower thickness, regardless of time point and ankle angle. Maximal ankle plantarflexion angles and corresponding fascicle lengths were lower on the injured ankle compared to the contralateral throughout rehabilitation. When normalized to the overall ankle ROM, both injured fascicles and MTJ displacement exhibited a comparably lower change in length when the ankle was passively rotated. These results indicate that when both ankles are passively exposed to the same ROM following ATR surgery, both ipsilateral Achilles tendon and gastrocnemius muscle fascicles exhibit limited lengthening compared to the contralateral MTU tissues. This appears to be consistent throughout the rehabilitation of gait, suggesting that current post-operative rehabilitative exercises do not appear to induce muscle adaptations in the affected MTU.

Keywords: muscle-tendon unit, achilles tendon rupture, ultrasonography, ultrasound, rehabilitation, achilles tendon – injuries

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INTRODUCTION

Though the incidence of Achilles tendon rupture (ATR) has been steadily increasing (Lantto et al., 2014), its treatment remains unclear (Chiodo et al., 2010). Following injury, ATR patients exhibit lasting functional deficits in plantarflexor strength (Mullaney et al., 2006; Metz et al., 2009; Olsson et al., 2011; Agres et al., 2015; Heikkinen et al., 2017b) and in athletic performance (Amin et al., 2013; Trofa et al., 2017). Such deficits in function are likely due to lasting structural changes in both tissues of the affected plantarflexor muscle-tendon unit (MTU) (Heikkinen et al., 2017b), which presents with a longer tendon length (Kangas et al., 2007; Rosso et al., 2013; Agres et al., 2018) and triceps surae muscle atrophy, regardless of either surgical or conservative treatment (Rosso et al., 2013).

The introduction of loading on the MTU through rehabilitative exercise has been proposed as a means to induce adaptations in the constituent tissues immediately following injury. Such early interventions appear to have an impact on functional outcomes (Holm et al., 2015) and have been effective in improving MTU tissue properties, for example, in the tendons of healthy participants (Arampatzis et al., 2010; Bohm et al., 2014), and in the muscle architecture of elderly patients following hip surgery (Suetta et al., 2008). Weight bearing soon after initial ATR treatment has been seen as an ideal rehabilitative treatment for patients (Brumann et al., 2014). However, various forms of exercise and mobilization interventions in ATR rehabilitation do not appear to significantly improve functional outcomes (Suchak et al., 2008; Agres et al., 2018; Eliasson et al., 2018; Kastoft et al., 2019), particularly with regards to the recovery of plantarflexor strength on the affected limb. Considering the main contribution of the triceps surae muscle to plantarflexion performance, comparably limited work has investigated structural changes in the triceps surae muscles following ATR.

Adaptations in muscle generally occur at a much faster rate than those in tendon when exposed to the same loads (Mersmann et al., 2014). In particular, muscle remodeling following ATR injury is rapid compared to the rate of tendon healing, which can last as long as 1 year (Sharma and Maffulli, 2005). Side-to-side differences in muscle morphology are apparent soon after ATR, as Hullfish and colleagues have recently reported that medial gastrocnemius fascicles on the ATR-injured side remain both shorter and more pennate within 1 month post-injury (Hullfish et al., 2019). Recent findings suggest that further muscle remodeling occurs after this time, with increases in medial gastrocnemius volume found between 6 and 26 weeks after ATR (Eliasson et al., 2018). It remains unclear if immediate side-to-side differences in muscle architecture persist in later stages of rehabilitation, particularly when patients regain independent, unassisted gait.

During gait, the MTU and its constituent tissues undergo various length changes, which ultimately affect and determine their capacity for force production. In particular, medial gastrocnemius fascicles show an active shortening-stretch-shortening cycle during the stance phase of walking in healthy subjects, which suggest that sarcomeres can operate near its optimal length. During gait, the majority of the stance phase (about 15–75%) is dedicated to fascicle lengthening (Ishikawa et al., 2007). Once fascicle lengthening has peaked, the gastrocnemius muscle is then activated to provide plantarflexor force to the ankle.

As a result, the lengthening capacity of muscle fascicles plays a major role in force production and overall MTU function. Within the context of ATR, an increased AT length implies that the overall operating length of the muscle in series, and consequently also sarcomere length, is comparably shorter than uninjured MTUs (Suydam et al., 2015). This, combined with increased compliance in the healing tendon (Wang et al., 2013; Wulf et al., 2017; Agres et al., 2018), suggests that the triceps surae muscle fascicles on the ATR injured side are exposed to different length changes compared to healthy fascicles.

Thus, the primary aim of this study was to determine the effect of passive sagittal ankle motion on relevant structures of the post-ATR MTU, namely muscle architecture parameters and AT length, during the mid-to-late stages of rehabilitation. To achieve this, the injured and contralateral structures of both muscle and tendon in ATR patients were assessed in maximal plantarflexion and dorsiflexion at 8, 12, and 16 weeks post-surgery. We hypothesized that throughout the rehabilitative period, the muscle fascicles on the ATR-injured MTU would demonstrate lower fascicle elongation when exposed to passive lengthening compared to the contralateral side. Furthermore, we hypothesized that this would be accompanied by a decreased AT elongation on the ATR-injured leg, as determined by displacements of the musculotendinous junction (MTJ). Finally, we postulated that when exposed to the same passive motion, relative changes in muscle thickness and pennation angle on the ATR-injured muscle would be lower than compared to the contralateral side.

MATERIALS AND METHODS

Patients

The present work was part of a larger experimental prospective study that aimed to determine the effect of a specific percutaneous surgical repair of ATR on overall function. A subset of 10 participants (46 ± 10 years old; BMI: 27.2 ± 3.9 kg/m^2; 1 female) were prospectively recruited after receiving percutaneous surgical repair using the Dresden instrument (Amlang et al., 2006) by a single surgeon within 7 days of initial injury. This cohort was assessed at 8, 12, and 16 weeks after surgical treatment of an acute, unilateral ATR (**Figure 1D**). Patients were included if classified as a Type 2a or 2b rupture after clinical ultrasound assessment of the initial injury (Amlang et al., 2011). Exclusion criteria included immobilized patients, open ATR surgery, non-surgical ATR treatment, concomitant injury to either the ipsilateral or contralateral lower extremity within 6 months of the ATR, re-rupture of the AT, and contralateral ATR. Patients received percutaneous surgery within 1 week of ATR injury. All patients gave informed consent prior to participation. The local ethics committee approved this study (EA/2/095/11) and all protocols were developed in accordance with the Declaration of Helsinki.

Due to technical problems with one data set at the 8-week follow-up, there was only a total of 9 patient data sets available for analysis for the initial time point. Furthermore, due to two drop-outs for the last time point, only 8 data sets were available for the 16-week follow-up.

Measurement Setup

A set of seven infrared motion capture cameras (MX F20, f = 250 Hz, VICON, Oxford, United Kingdom) was used to determine the ankle angles from each limb. Markers were placed on the medial and lateral condyles of the femur, medial and lateral malleoli, the 1st head of the metatarsal, the calcaneus, the lateral aspect of the thigh, and the greater trochanter. These marker positions were used to determine the ankle angle relative to a reference position, whereby the foot was in a neutral position, where motion capture data was collected. Simultaneous non-invasive assessment of the medial gastrocnemius muscle architecture and medial gastrocnemius MTJ was performed using a 10 cm, 7.5 MHz B-mode ultrasonography probe (f = 25 Hz, MyLab60, Esaote S.p.A., Genoa, Italy) while patients were seated with the knee outstretched and the ankle placed on a dynamometer pedal. Each ankle then was slowly brought to maximal plantarflexion and dorsiflexion, based on subjective feedback from the participant as to when pain or mobility limits were reached. The pedal was then held in place at this angle and paused. Once each of these positions was reached, both motion capture data and two ultrasound images were collected and synchronized (**Figure 1A**).

The first ultrasound image recorded medial gastrocnemius (GM) architectural parameters at the midbelly of the medial gastrocnemius (**Figure 1B**). The second image recorded the location of the musculotendinous junction (MTJ) of the insertion of the medial gastrocnemius into the Achilles tendon. For the second image, the relative displacement of the MTJ was determined by calculating the MTJ distance between plantarflexion and dorsiflexion, with reference to a small strip of tape directly placed on the skin. This functioned as an externally placed echo-absorbing skin marker, which remained unmoved for both measurement positions (**Figure 1C**). Furthermore, the rest length of the Achilles tendon on both sides was determined from the MTJ to the calcaneus with the patient in 20° of plantarflexion and an outstretched knee, due to the slack in the muscle-tendon unit at this position (De Monte et al., 2006).

Image Analysis

Following collection of all images, the fascicle length, pennation angle, and thickness of the muscle were measured using open-source software (ImageJ 1.48v, National Institutes of Health, Bethesda, MD, United States) and a stylus-equipped monitor (Wacom Cintiq 21UX DTK-2100, Portland, OR, United States). Fascicle length was defined as the linear distance between aponeurosis insertions. Pennation angle was measured between the deep aponeurosis and the fascicle. Muscle thickness was measured as the shortest linear distance between the upper and lower aponeuroses (**Figure 1B**). Due to variations in muscle architecture along its length, three measurements of each parameter were measured and averaged for each captured image. To avoid bias, images were randomized and blinded for the injured side during analysis.

To account for possible side-to-side differences in ankle sagittal plane mobility, all parameters gathered from image analysis were also normalized to the ankle range of motion (ROM). To describe this in detail: for a given parameter, p, the value at plantarflexion was subtracted from the corresponding value at dorsiflexion, yielding the absolute change of parameter, or Δp. This quantity was calculated for each leg type, or side (Δp_{side}), and then normalized to the ROM corresponding side (ROM_{side}). This results in ROM-normalized parameters, as described by the formula

$$\frac{\Delta p_{side}}{ROM_{side}} = \frac{p_{side\ (dorsiflexion)} - p_{side\ (plantarflexion)}}{\theta_{side\ (dorsiflexion)} - \theta_{side\ (plantarflexion)}}$$

where p refers to the parameter calculated (fascicle length, pennation angle, or muscle thickness), *side* refers to the limb of interest (ATR injured or contralateral), and θ refers to the ankle angle in degrees.

Statistics

Means, standard deviations, and 95% confidence intervals were calculated for all parameters from each patient at each time point. All parameters were tested for normality using Shapiro-Wilk, then for sphericity using Mauchly's test in SPSS (IBM, Armonk, NY, United States). A factorial repeated-measures analysis of variance (ANOVA) was performed to analyze differences with consideration of two main effects: the time of measurement post-surgery (8, 12, or 16 weeks) and the leg type (ATR injured or contralateral). If sphericity could not be assumed according to Mauchly's test, a Greenhouse-Geisser correction was applied to the factorial repeated-measures ANOVA. A Bonferroni adjustment was applied to the original level of significance ($\alpha = 0.05$) due to the multiple comparisons performed. Thus, the final level of significance was set at $\alpha = 0.01$ ($\alpha = 0.05/5$). When significant contrasts were found, the effect size was calculated for the relevant pair and interpreted according to Cohen (1988).

In order to determine the intra-rater reliability of ultrasound image processing, an experienced investigator blinded to the patient type and injured side assessed all parameters for the dorsiflexion position in two separate analysis sessions. A two-way mixed-effects intraclass correlation coefficient (ICC) for absolute agreement in single measures (McGraw and Wong, 1996) was calculated for each of the three measured parameters.

RESULTS

The absolute values of all parameters are listed in **Table 1** for the included subjects where data was available for all time points (n = 8) for reference. There was no significant effect of time of measurement post-surgery on any of the investigated parameters. Thus, the results below detail findings from the factorial repeated-measures ANOVA with regards to the main effect of leg type, as well as the interaction of time of measurement with leg type. These are presented with means, significance values, effect sizes (for significant findings), and 95% confidence

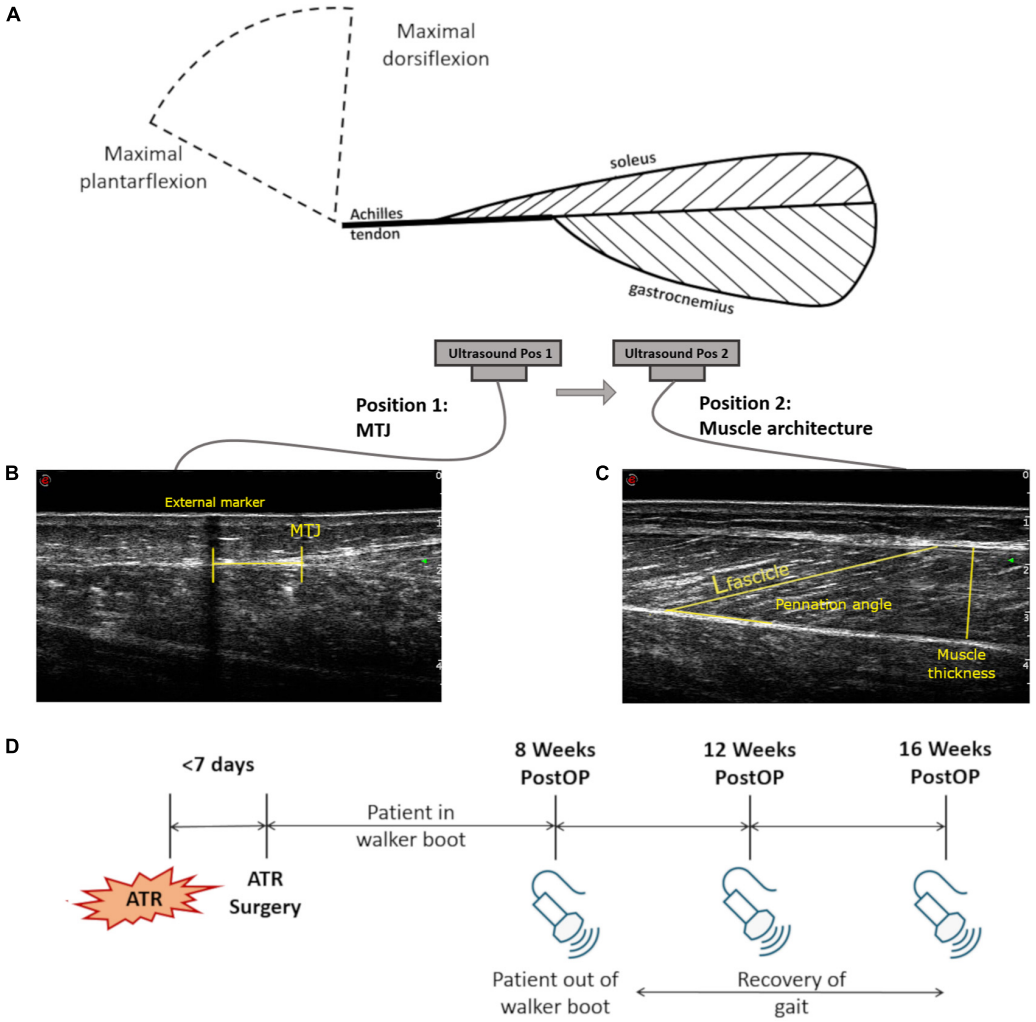

FIGURE 1 | Measurement setup and study description. **(A)** Schematic of the measurement setup for the ultrasound capture, showing the muscle-tendon unit (MTU) tissues and the two positions of the ankle and the two relative positions of the single ultrasound probe. Two separate ultrasound images were taken at both maximal plantarflexion and dorsiflexion: **(B)** Sample ultrasound image locating the musculotendinous junction (MTJ) displacement of the Achilles tendon to the medial gastrocnemius with respect to an external skin marker. **(C)** Sample ultrasound image of medial gastrocnemius architecture measures at the midbelly of the calf, illustrating how fascicle length ($L_{fascicle}$), muscle thickness, and pennation angle were assessed. **(D)** Timeline of the three measurement sessions with relation to the time of ATR injury and surgical treatment.

intervals for the difference of the ATR-injured side (INJ) from the contralateral side (CON).

Rest Tendon Length

The Achilles tendon length at 20 degrees of plantarflexion was significantly longer on INJ compared to CON (20.6 vs 18.6 cm, $p = 0.004$, CI [3.0 to 35.7] cm) during the rehabilitation period. However, no interaction effects were found between the main effects of leg type and time point.

Parameters at Maximal Plantarflexion

The measured ankle angle at maximal plantarflexion was found to be significantly lower on INJ compared to CON (**Table 1**, 26.8 vs 35.1°, $p = 0.002$, $r = 0.91$, CI [−12.0 to −4.5]°). When the foot was held in maximal plantarflexion, there was a significant main effect of leg type on medial gastrocnemius thickness (**Figure 2A**, 17.3 vs 20.6 mm, $p = 0.002$, $r = 0.90$, CI [−4.8 to −1.8] mm) and on muscle fascicle length (38.2 vs 42.0 mm, $p = 0.023$, CI [−8.1 to 0.55] mm). Both parameters were lower on on INJ compared to CON. Pennation angle (25.5 vs 26.9°, $p = 0.136$, CI [−3.55 to 0.62] °) did not exhibit any significant effects of leg type at maximal plantarflexion. Furthermore, no interaction effects were found between time of measurement and leg type at maximal plantarflexion.

Parameters at Maximal Dorsiflexion

The measured ankle angle at maximal dorsiflexion did not exhibit any effects of leg type at maximal dorsiflexion (**Table 1**, −8.0 vs −5.6°, $p = 0.102$, CI [−5.39 to 0.64] °). At

TABLE 1 | Absolute values of ankle angles and muscle architecture parameters on the ATR injured and contralateral (CON) musle-tendon units for included subjects (n = 8) at two ankle positions at three different measurement time points during the rehabilitation period.

		Ankle position			
		Maximum plantarflexion		Maximum dorsiflexion	
Parameter	Time post-op	ATR	CON	ATR	CON
Ankle angle (°)	8 weeks	26.8 ± 5.9	31.9 ± 2.7	−4.9 ± 6.0	−8.1 ± 5.2
	12 weeks	29.3 ± 7.2	37.1 ± 5.8	−7.8 ± 8.0	−4.3 ± 6.5
	16 weeks	26.8 ± 3.7	35.4 ± 5.1	−10.0 ± 2.9	−4.4 ± 5.0
Fascicle length (mm)	8 weeks	38.1 ± 2.3	39.4 ± 4.1	49.0 ± 4.7	57.7 ± 4.0
	12 weeks	36.5 ± 5.9	46.0 ± 12	45.7 ± 5.8	59.9 ± 10.3
	16 weeks	35.6 ± 5.3	40.1 ± 7.6	41.9 ± 6.8	56.7 ± 7.9
Muscle thickness (mm)	8 weeks	17.9 ± 2.9	20.3 ± 2.7	15.4 ± 2.6	18.4 ± 2.2
	12 weeks	17.0 ± 2.4	20.7 ± 3.0	15.7 ± 3.0	18.6 ± 3.6
	16 weeks	16.8 ± 3.1	20.8 ± 3.5	15.2 ± 3.7	18.3 ± 3.5
Pennation angle (°)	8 weeks	22.0 ± 3.3	27.4 ± 3.5	19.3 ± 1.3	19.3 ± 2.2
	12 weeks	26.2 ± 6.8	24.8 ± 6.0	22.4 ± 5.9	19.9 ± 3.8
	16 weeks	28.6 ± 7.6	28.3 ± 5.9	22.3 ± 5.3	20.2 ± 2.5

Values are reported as means ± SD of the average values for all subjects. Ankle joint angle is defined with reference to a neutral ankle position set at 0°, where positive values indicate plantarflexion and negative values indicate dorsiflexion.

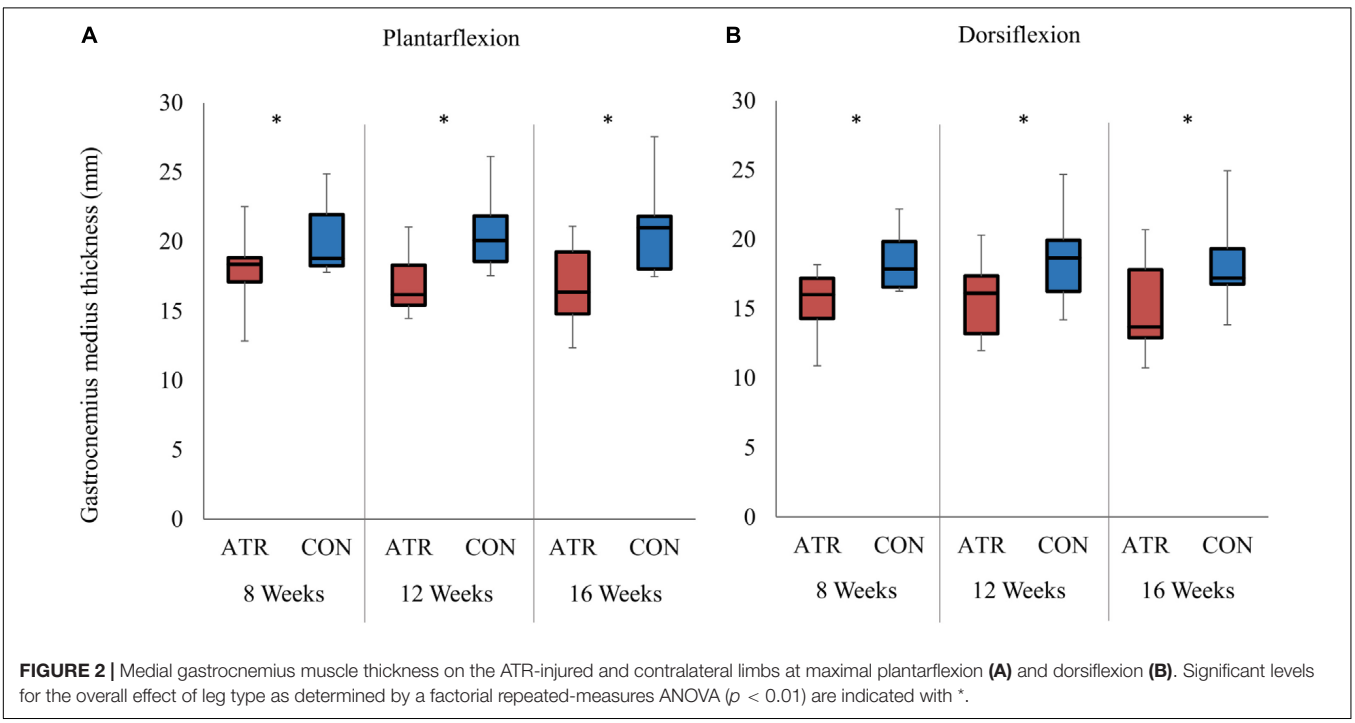

FIGURE 2 | Medial gastrocnemius muscle thickness on the ATR-injured and contralateral limbs at maximal plantarflexion **(A)** and dorsiflexion **(B)**. Significant levels for the overall effect of leg type as determined by a factorial repeated-measures ANOVA ($p < 0.01$) are indicated with *.

this dorsiflexed angle, there was a significant main effect of leg type on medial gastrocnemius thickness (**Figure 2B**, 15.4 vs 18.4 mm, p = 0.002, r = 0.90, CI [−4.5 to −1.5] mm), with INJ values lower than CON. Similarly, muscle fascicle length at maximal dorsiflexion also exhibited a significant main effect of leg type (45.5 vs 58.1 mm, $p < 0.001$, r = 0.98, CI [−15.0 to −10.1] mm). However, pennation angle (21.3 vs 19.9°, p = 0.188, CI [−0.92 to 3.76] °) did not exhibit any significant effects of leg type at maximal dorsiflexion. Furthermore, no interaction effects were found between time of measurement and leg type at maximal dorsiflexion.

Absolute Changes of Parameters in the Range of Motion

Ankle mobility, as determined by the ankle ROM, was found to be similar between INJ and CON, with no effect of leg type (34.8 vs 40.7°, p = 0.033, CI [−11.2 to 0.66] °, **Figure 3A**). However, the absolute change in relative MTJ displacement exhibited a

significant main effect of leg type ($p = 0.001$, $r = 0.94$), with INJ displacements lower than CON (**Figure 3B**). Absolute changes in muscle fascicle length similarly exhibited a significant main effect of leg type (8.8 vs 16.2 mm, $p < 0.001$, $r = 0.95$, CI [−9.9 to −5.0] mm, **Figure 3C**). Between maximal plantarflexion and maximal dorsiflexion, the change of muscle fascicle length on INJ was significantly lower than on CON. Both overall changes in medial gastrocnemius thickness (1.8 vs 2.1 mm, $p = 0.496$, CI [−1.3 to 0.7] mm) and pennation angle (−4.2 vs −7.0°, $p = 0.039$, CI [0.2 to 5.4] °) did not exhibit any significant effects of leg type.

There was a significant interaction between time of measurement and leg type for ankle ROM ($p = 0.003$). Contrasts were performed and revealed significant interactions when comparing INJ to CON for the 8 weeks time point and the 16 weeks time point ($p = 0.013$, $r = 0.83$), with INJ ROM increasing more with time compared to CON. The remaining contrast revealed no significant interaction term when comparing INJ to CON for the 12 weeks and the 16 weeks time point ($p = 0.505$, $r = 0.28$).

Normalized Changes of Parameters to the Range of Motion

Overall changes in muscle fascicle length, when normalized to overall ankle ROM, displayed a significant main effect of leg type (0.27 vs 0.47 mm/°, $p = 0.001$, $r = 0.93$, CI [−0.28 to −0.13] mm/°), with relative changes of INJ muscle fascicle length lower than CON (**Figure 4A**). Furthermore, overall MTJ displacement normalized to total ankle ROM also exhibited a significant main of leg type (0.38 vs 0.79 mm/°, $p = 0.001$, $r = 0.92$, CI [−0.58 to −0.23] mm/°) (**Figure 4B**). This ROM-normalized MTJ displacement on INJ was found to be lower than that of CON.

When the overall change in medial gastrocnemius thickness was normalized to ankle ROM, no effect of leg type was found (0.056 vs 0.053 mm/°, $p = 0.843$, CI [−0.031 to 0.037] mm/°). Similarly, ROM-normalized changes in pennation angle were found to be similar between INJ and CON sides (−0.13 vs −0.18, $p = 0.199$, CI [−0.037 to 0.14]). For all examined parameters, there was no interaction found between the main effects of the time of measurement post-surgery and leg type.

Image Analysis Reliability

Intra-rater reliability was found to be excellent for fascicle length (ICC = 0.955) and very good for pennation angle (ICC = 0.840) and MTJ displacement (ICC = 0.844). The intra-rater reliability of muscle thickness was good with an ICC value of 0.757.

DISCUSSION

This longitudinal study determined the effect of passive ankle motion on the architecture and structure of the component tissues of the MTU during the rehabilitative period after percutaneous surgical repair of an ATR, when compared to the uninjured contralateral side. Taking previous reports of increased AT length and compliance into consideration, it was hypothesized that the medial gastrocnemius muscle would exhibit lower fascicle elongation when exposed to passive lengthening, together with limited MTJ displacement. The results here support this hypothesis, indicating that when normalized to the available ROM in the ankle, both the tendon and the muscle fascicles consistently exhibit a lower capacity to passively lengthen on the ATR side when compared to the contralateral side post-surgery. However, the hypothesis postulating that relative changes in muscle thickness and pennation angle would be lower on the ATR-injured side were not supported within this work. These results indicate that when both ankles are passively exposed to the same ROM following ATR surgery, both ipsilateral Achilles tendon and gastrocnemius muscle fascicles exhibit limited lengthening compared to the contralateral MTU tissues (**Figure 5**). This appears to be consistent throughout the rehabilitation of gait in this particular cohort.

These findings imply that restoration of side-to-side passive ankle ROM, a common clinical benchmark, does not reflect the remaining side-to-side differences in passive fascicle lengthening following ATR surgery. Such limitations in fascicle lengthening on the ATR side may have multiscale implications on the affected muscle in parallel. Recent work investigating the effect of passive lengthening on gastrocnemius architecture in healthy subjects has determined that changes in fascicle length are comparable to length changes in muscle belly length (Bolsterlee et al., 2017). Passive changes in fascicle length may also be indicative of changes at the sarcomere level, as observed in multiscale *in vivo* investigations of passive fascicle and sarcomere length in the tibialis anterior muscle (Lichtwark et al., 2018). Based on the observations at the fascicle level within this study, passive sarcomere-level length changes may also be limited on the ATR side compared to the contralateral side. Overall, this suggests that following ATR surgery, the sarcomeres on the ATR side operate on a different portion of the length-tension relationship compared to the contralateral side, even if there are no side-to-side differences in ankle ROM.

Throughout the rehabilitation period, ATR muscle fascicles consistently exhibited a shorter length through the available ROM compared to the contralateral side (**Figure 3C**). This was accompanied by limited MTJ displacement throughout the same ROM (**Figure 3B**). These results are comparable to previous short-term measurements that found consistently shorter fascicle lengths and lower muscle thickness up to 4 weeks post-ATR when held in plantarflexion (Hullfish et al., 2019). This appears to be consistent throughout the rehabilitation of gait, suggesting that current post-operative rehabilitative exercise do not appear to induce muscle adaptations on fascicle length in the affected MTU.

The results within this work suggest that limited to no muscle hypertrophy occurs in the medial gastrocnemius during rehabilitation between 8 and 16 weeks. This is in contrast with findings from other work. In particular, pennation angle was not observed to significantly change with time within this study, which contrasts short-term findings from Hullfish and colleagues, who found significant increases within 1 month post-ATR. Surprisingly, within the time frame investigated, muscle fascicle length did not significantly change, which explains no changes in muscle thickness on the injured muscle during the rehabilitative period. These results are in contrast with other follow-ups of

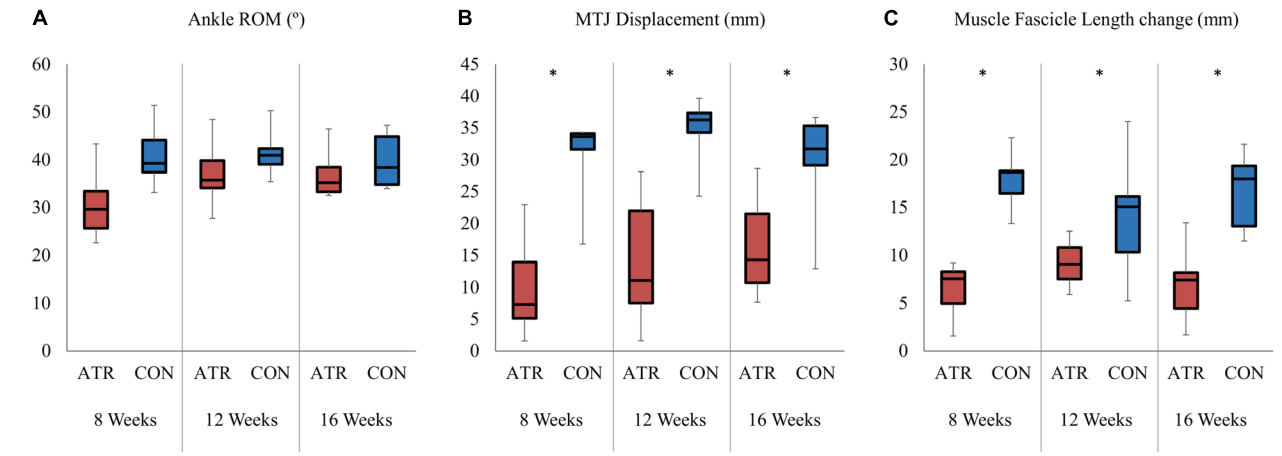

FIGURE 3 | Absolute changes of selected parameters between maximal plantarflexion and maximal dorsiflexion for both the on the ATR-injured and contralateral (CON) sides at each measurement point. Significant levels for the overall effect of leg type as determined by a factorial repeated-measures ANOVA ($p < 0.01$) are indicated with *. **(A)** Range of motion of the ankle (°). **(B)** Absolute displacement of the musculotendinous junction (MTJ, in mm). **(C)** Absolute change in the medial gastrocnemius fascicle length (mm).

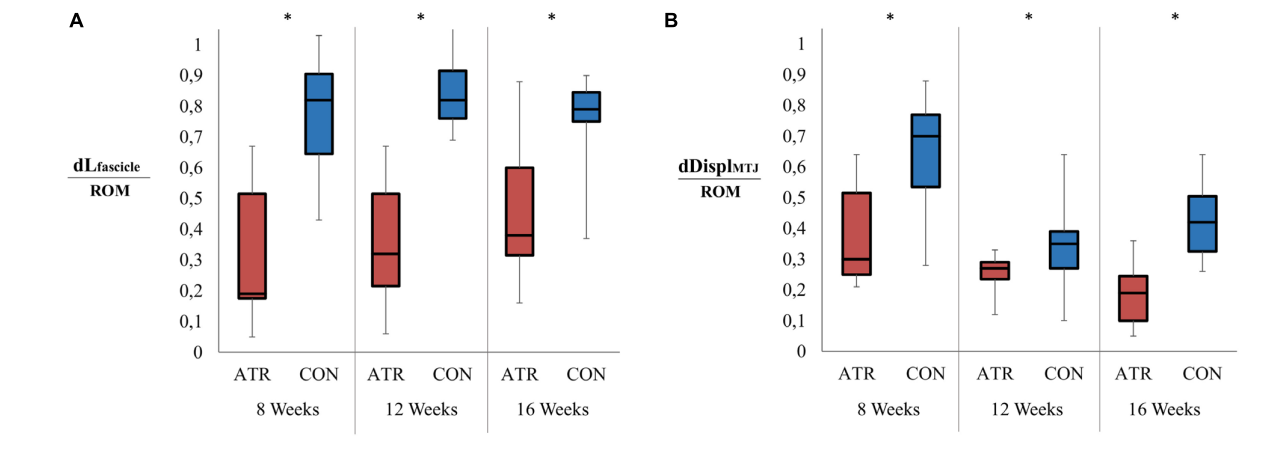

FIGURE 4 | Ranges in relevant muscle-tendon unit architecture parameters between maximal plantarflexion and maximal dorsiflexion, normalized to the overall ankle range of motion (ROM). Significant levels for the effect of leg type [as determined by a factorial repeated-measures ANOVA ($p < 0.01$)] are indicated with *. **(A)** Relative change in the medial gastrocnemius fascicle length (mm) as normalized to the ankle range of motion (°). **(B)** Displacement of the musculotendinous junction (MTJ, in mm) as normalized to the ankle range of motion (°).

volumetric changes in these muscles in ATR patients, which found that the medial gastrocnemius significantly increased in cross-sectional area between 6 and 26 weeks post-ATR (Eliasson et al., 2018). A possible explanation for this may lie in the method that muscle was assessed. There are considerable limitations in the two-dimensional assessment of muscle using ultrasound, particularly since transducer position can affect measures such as fascicle length (Bolsterlee et al., 2016). Though recent work suggests that there is a correlation between two-dimensional muscle thickness and MRI-measured cross-sectional area in athletes (Franchi et al., 2018), this may not hold true within the context of pathologies such as ATR.

The results here indicate that during the mid- to late-stages of rehabilitation after ATR surgery, when patients are removed from assistive devices and begin to ambulate independently, that adaptive behavior in the medial gastrocnemius muscle on the ATR side is limited compared to the uninjured contralateral side. This is in stark contrast to what was observed in the patients themselves in day-to-day activities like gait, as all subjects were fully mobile in daily activities by 16 weeks. This mismatch between the lack of difference in overall ankle mobility and sustained physiological deficits at the MTU-level further support the theory that compensatory action by neighboring muscle groups, such as the flexor hallucis longus (Finni et al., 2006; Heikkinen et al., 2017a), may be ultimately responsible for the recovery of the ATR patient. However, these compensatory actions are unable to restore previous function, particularly in high-level sports (Amin et al., 2013). This suggests that current rehabilitative exercise measures performed within this period of ATR rehabilitation (specifically,

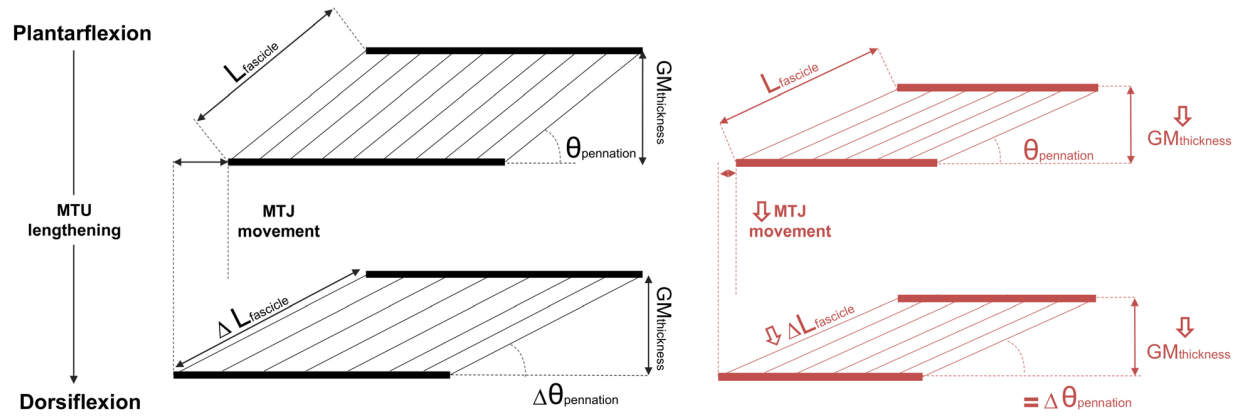

FIGURE 5 | A schematic summarizing the observed differences in MTU tissues when exposed to passive MTU lengthening between the contralateral (CON, left column, black) and ATR-injured tissues (right column, red) after surgical ATR repair. The top row depicts the two muscles as seen in plantarflexion, when the MTU is comparably slack, and the bottom row depicts the two muscles as seen in dorsiflexion, when the MTU is held in more tension. Absolute differences are indicated in the bottom row with Δ ahead of the parameter, as the difference between plantarflexion and dorsiflexion. The top left diagram depicts the parameters of interest on the contralateral side: $L_{fascicle}$ is the measured length of the medial gastrocnemius fascicle, $\theta_{pennation}$ denotes the pennation angle, $GM_{thickness}$ denotes the measured thickness of the medial gastrocnemius, and the distance between the dashed lines indicates the relative movement of the musculotendinous junction (MTJ). Arrows on the right column ATR parameters indicate significant differences to CON at the same position. Equal signs indicate no significant differences compared to CON.

recovery of independent gait) may not induce adaptations in the targeted MTU muscles, but rather allow for the development of compensatory adaptations for disadvantages in the affected triceps surae muscle.

These results further imply that a combination of increased rest tendon length and increased tendon compliance following surgical ATR treatment negatively affects passive force transmission between the two constituent tissues of the muscle-tendon unit. This directly impacts the efficacy of active force transmission from the ATR-side muscle to the tendon in order to actively plantarflex the ankle. To potentially compensate for these side-to-side morphological differences, contractile force in the gastrocnemius could be increased by augmenting the activation of the muscle itself. Longer-term follow ups in ATR patients indicate that both the lateral and medial gastrocnemius exhibit higher integrated EMG activity on the ATR side compared to the contralateral side during gait, as found in patients that similarly exhibited longer Achilles tendon rest length on the ATR side (Suydam et al., 2015). Yet despite these modulations at the neuromuscular level, they ultimately cannot overcome the negative morphological changes in the muscle. Here, the ATR-affected gastrocnemius appears to both have a shorter overall length within the MTU and lower thickness compared to the contralateral limb, suggesting overall volumetric atrophy and further reducing the MTU's capacity for active plantarflexion. The findings presented here, when placed in context of previous findings, suggest that negative adaptations in the morphology of both MTU constituent tissues following ATR aggregate, ultimately leading to limited passive force transmission between the muscle and tendon, which in turn limits the MTU's capacity to actively plantarflex the ankle.

A limitation of this work is that only the AT was considered within the context of the tendinous structures, and no investigations of the aponeuroses were performed. These results do not exclude the possibility that changes in elasticity could also affect the deep and superior aponeuroses of the gastrocnemius muscle. Increased lengthening of the entire MTU leads to limited deformation in the Achilles tendon in series, but this does not exclude the possibility that components of the parallel elastic component have also become more compliant. Another limitation is that for all measured positions, the knee remained outstretched and, thus, the plantarflexed position may not represent a perfectly slack MTU rest position, with the knee flexed and the ankle neutral, both at 90° (Grieve et al., 1978). However, slackness in the MTU has been shown to be present at similar knee and ankle angles as was measured here (De Monte et al., 2006). Thus, we are confident in our measurement positions of the MTU for the plantarflexed position as representative of a slack MTU. A further limitation of this study is the subjective method used to assessed the limits of ankle ROM, which was determined based on the subject's self-reported pain and discomfort while seated in the dynamometer. This method was chosen for the safety and security of the patient during measurement sessions. As pain thresholds vary from subject to subject, this introduces variability in the ROM measurements. However, considering most reports of passive ROM often use manual goniometers to perform measurements, we feel more confident in our use of motion capture to determine this parameter.

A final limitation of this study is the lack of a healthy control comparison group to compare to both limbs of the ATR injured group, which were not available within the context of this initial study. Previous existing studies in healthy participants have

already extensively investigated how passive changes in ankle angle affect muscle fascicle length at different locations of the muscle belly using multiple imaging modalities (Herbert et al., 2002, 2011; Bolsterlee et al., 2017). The results from this work, however, give a first indication that within-subject differences are present, even within this small cohort. Future studies should aim to include matched controls to ATR patients, to determine if adaptations on both INJ and CON muscle fascicles are distinct from those in uninjured, healthy participants.

CONCLUSION

This study shows that following ATR surgery and during rehabilitation, the muscle fascicles of the affected MTU exhibit a limited capacity to lengthen when the ankle is passively moved into dorsiflexion when compared to the contralateral side. This is coincident with limited passive AT lengthening on the affected MTU, as measured by MTJ displacement. Furthermore, these altered passive properties are accompanied by sustained medial gastrocnemius atrophy, as measured by muscle belly thickness, as well as a longer Achilles tendon, as measured at rest. These collective results indicate that both tendon and muscle alter their passive structural properties soon after ATR and remain this way, even during the introduction of more rehabilitative exercise and movement. Furthermore, when both ankles are passively exposed to the same ROM following ATR surgery, both ipsilateral Achilles tendon and medial gastrocnemius muscle fascicles exhibit limited lengthening compared to the contralateral MTU tissues. Our findings indicate that current rehabilitative exercises that aim to improve plantarflexor muscle strength may not effectively target the muscle tissues of the affected MTU in the short- to mid-term following surgical repair of an ATR. This appears to be consistent throughout the rehabilitative period, suggesting that current post-operative rehabilitative exercise do not appear to induce muscle adaptations in the affected MTU following surgery. The results here imply that impaired passive force transmission on the ATR side likely stems from negative morphological adaptations in both the affected tendon and the muscle tissues. Further investigation of rehabilitative exercise interventions within the context of muscle adaptation after surgical ATR repair are warranted.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

All study protocols were reviewed and approved by Ethikkommission der Charité-Universitätsmedizin Berlin. The patients provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ANA, AA, SM, and GD designed the experimental study. SM performed surgeries. SM and TG recruited participants. ANA and TG performed the data collection. ANA, AA, and GD interpreted the results and drafted the manuscript. SM and TG contributed to the revision of the text. All authors contributed to the article and approved the submitted version.

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The Free Achilles Tendon Is Shorter, Stiffer, Has Larger Cross-Sectional Area and Longer T2* Relaxation Time in Trained Middle-Distance Runners Compared to Healthy Controls

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Tendon geometry and tissue properties are important determinants of tendon function and injury risk and are altered in response to ageing, disease, and physical activity levels. The purpose of this study was to compare free Achilles tendon geometry and mechanical properties between trained elite/sub-elite middle-distance runners and a healthy control group. Magnetic resonance imaging (MRI) was used to measure free Achilles tendon volume, length, average cross-sectional area (CSA), regional CSA, moment arm, and T2* relaxation time at rest, while freehand three-dimensional ultrasound (3DUS) was used to quantify free Achilles tendon mechanical stiffness, Young's modulus, and length normalised mechanical stiffness. The free Achilles tendon in trained runners was significantly shorter and the average and regional CSA (distal end) were significantly larger compared to the control group. Mechanical stiffness of the free Achilles tendon was also significantly higher in trained runners compared to controls, which was explained by the group differences in tendon CSA and length. T2* relaxation time was significantly longer in trained middle-distance runners when compared to healthy controls. There was no relationship between T2* relaxation time and Young's modulus. The longer T2* relaxation time in trained runners may be indicative of accumulated damage, disorganised collagen, and increased water content in the free Achilles tendon. A short free Achilles tendon with large CSA and higher mechanical stiffness may enable trained runners to rapidly transfer high muscle forces and possibly reduce the risk of tendon damage from mechanical fatigue.

Keywords: free Achilles tendon, magnetic resonance imaging, freehand three-dimensional ultrasound, trained middle-distance runners, geometry, Young's modulus, T2* relaxation time

INTRODUCTION

The Achilles tendon stores and recovers strain energy to improve mechanical energy generation-absorption of the triceps surae muscles and reduce metabolic cost during dynamic activities (Alexander and Bennet-Clark, 1977; Roberts et al., 1997; Lichtwark and Wilson, 2005; Wiesinger et al., 2017). *In vivo* studies have shown that Achilles tendon geometry and/or mechanical properties adapt to long-term mechanical loading in a manner that is specific to the type and duration of the applied loads (Bohm et al., 2015; Wiesinger et al., 2015, 2016). These long-term adaptations are believed to enhance the mechanical function of the Achilles tendon and triceps surae complex and keep the strain experienced by the Achilles tendon within physiological limits (Ker et al., 1988; Bohm et al., 2015; Wiesinger et al., 2015, 2016).

Rosager et al. (2002), Magnusson and Kjaer (2003), and Wiesinger et al. (2016) reported 15, 30, and 36% larger non-normalised cross-sectional area (CSA) in male runners compared to non-runners, respectively. Similar differences are also reported between runners and non-weight bearing athletes (e.g., kayakers; Kongsgaard et al., 2005), and in older endurance runners versus older non-runners (+16%) and younger non-runners (+30%; Stenroth et al., 2016). While free Achilles tendon CSA tends to be higher in habitual runners (Rosager et al., 2002; Magnusson and Kjaer, 2003), a recent review suggests there is limited evidence to support a concomitant increase in Achilles tendon mechanical stiffness and/or Young's modulus (Wiesinger et al., 2015). However, unlike CSA, which is commonly measured from free Achilles tendon region, mechanical properties are often measured from the gastrocnemius muscle-tendon junction to the calcaneus (Lichtwark and Wilson, 2005; Bohm et al., 2014; Wiesinger et al., 2016), which also includes the aponeurosis. Thus, understanding the adaptation of the free Achilles tendon requires both geometry and mechanical properties of the free portion of the tendon (from the soleus muscle-tendon junction to the calcaneus) to be assessed independently of the aponeurosis. While magnetic resonance imaging (MRI) and freehand three-dimensional ultrasound (3DUS) methods have been used to assess resting tendon geometry (Devaprakash et al., 2019), freehand 3DUS has the added advantage over MRI of allowing tendon geometry to be assessed in the laboratory environment at rest and under load (Obst et al., 2014a; Nuri et al., 2017), so that the mechanical properties of the tendon can also be evaluated.

Ultrashort echo time (UTE) based MRI methods have been used to capture average $T2^*$ relaxation time of the Achilles tendon (mid-portion, insertion region, muscle-tendon junction, and whole Achilles tendon-aponeurosis) in a group of recreational long distance runners compared to healthy volunteers, showing longer $T2^*$ relaxation times in the mid-portion and whole Achilles tendon-aponeurosis for runners (Grosse et al., 2015). Longer $T2^*$ relaxation times are believed to reflect a greater amount of free water protons between collagen fibres, reduced collagen alignment, and have previously been reported to distinguish between healthy and pathological Achilles tendons (Juras et al., 2012, 2013; Gärdin et al., 2016; Qiao et al., 2017). However, it remains unclear if $T2^*$ relaxation time, which is indicative of collagen organisation/disorganisation (Juras et al., 2013; Gärdin et al., 2016), is related to Young's modulus in the free Achilles tendon. Any such relationship would be expected to depend on the sensitivity of $T2^*$ relaxation time to fluid alterations compared to alterations in collagen disorganisation, as well as the extent to which collagen disorganisation is sufficient to influence the bulk tissue modulus.

The purpose of this study was to use MRI and freehand 3DUS to: (1) compare free Achilles tendon geometry, mechanical stiffness, Young's modulus, length normalised mechanical stiffness, and $T2^*$ relaxation time between trained elite/sub-elite middle-distance runners and a healthy control group; and (2) determine the relationship between $T2^*$ relaxation time and Young's modulus of the free Achilles tendon. Given the strong existing evidence showing that the free Achilles tendon CSA of runners tends to be higher than non-runners (Rosager et al., 2002; Magnusson and Kjaer, 2003; Wiesinger et al., 2016), it was hypothesised that trained runners would have a larger free Achilles tendon CSA resulting in greater free Achilles tendon mechanical stiffness. Based on the findings of Grosse et al. (2015), $T2^*$ relaxation times were also expected to be higher in runners compared to a healthy control group.

MATERIALS AND METHODS

Participant Characteristics

Sixteen trained elite/sub-elite middle-distance runners (10 males and 6 females) and 16 healthy controls (11 males and 5 females) with no prior history or symptoms of Achilles tendon injury participated in the study. Trained runners performed more than 80 km of running per week as part of their training, competed regularly at state/national/international level competitions, and had no recent or recurrent Achilles tendon or lower limb injuries as assessed by an experienced physician. Trained runners with symptomatic Achilles tendinopathy as determined via clinical assessment (Maffulli et al., 2003; Scott et al., 2020) and ultrasound imaging (brightness mode and microbubble technique) were excluded. The study was approved by the relevant institutional Human Research Ethics Committees and all participants provided written informed consent prior to participation in the study.

Experimental Design and Data Collection Protocol

Data collection was performed at two sites (Trained runners: Australian Institute of Sport, Canberra, Australia; Healthy controls: Griffith University, Queensland, Australia) due to the inability to test participants from both groups at a single testing centre. Participants attended two experimental testing sessions. During the first session, MRI scans (anatomical and UTE $T2^*$ relaxation time) of the participants' free Achilles tendon were obtained (Trained runners: Universal Medical Imaging, ACT, Australia; Healthy controls: QSCAN Radiology Clinics, Queensland, Australia). In the second session, freehand 3DUS scans were performed at rest and under load in order to determine free Achilles tendon mechanical stiffness, Young's modulus, and length normalised mechanical stiffness.

The freehand 3DUS set-ups at the two experimental testing sites were identical. Participants refrained from strenuous physical activity in the 24 h before each experimental session. Prior to each session, all participants completed a preconditioning protocol that involved walking at a self-selected pace (~270 gait cycles; Hawkins et al., 2009). All measures were obtained from the participants' preferred/dominant leg. Free Achilles tendon geometry of trained middle-distance runners recorded during rest using MRI and freehand 3DUS has been partly presented in Devaprakash et al. (2019).

Magnetic Resonance Imaging

Anatomical MRI scans of the distal lower limb (i.e., including distal tibia-fibula and foot) were acquired on a Philips Ingenia 3.0 Tesla scanner (Ingenia 3.0 T, MR system, Philips medical systems, Amsterdam, North Holland, Netherlands) using an eight-channel ankle coil (ACT, Queensland: PDW 3D TSE, TR/TE 1000/41 ms) with the participant lying in a supine position with the hip in neutral position, the knee fully extended, and the ankle in a neutral position (0° dorsiflexion). Slice thickness, slice gap, and resolution were 0.6, 0.3, and 0.27 mm, respectively. MRI scans were acquired in the sagittal plane and exported in DICOM format for further processing.

With the participant's ankle positioned at approximately 30° plantarflexion, UTE T2* relaxation time sequence (Fast Field Echo) was acquired using a 16-channel knee coil to assess tissue quality. Imaging parameters for obtaining UTE T2* data at both MRI centres: (1) relaxation time (TR; ACT, Queensland: 14.674 and 16.481 ms), (2) echo time (TE; ACT, Queensland: 0.18, 2.50, 4.82, 7.14, 9.46 and 0.21, 1.86, 3.51, 5.15, 6.80, 8.45, 10.09 ms), (3) flip angle (ACT, Queensland: 10°), and (4) image matrix (ACT, Queensland: 256 × 256). UTE T2* sequence data were exported in PAR/REC (Queensland) and XML/REC (ACT) formats.

Anatomical MRI data stored in DICOM format were converted to Stradwin files (version 5.4, Medical imaging group, University of Cambridge, Cambridge, England, UK). Following this, MRI data were resliced at one-pixel resolution (0.27 mm) in the transverse plane (Treece et al., 2003) and sparse manual segmentation of the free Achilles tendon was performed by the same experimenter (DD, unblinded to the participant's identity) in the transverse plane at uniform space intervals (6–8 slices per tendon) for all participants. Free Achilles tendon insertion at the calcaneal notch and the soleus muscle-tendon junction were defined as distal and proximal end of the free Achilles tendon. Free Achilles tendon 3D reconstruction was performed using sparse contour interpolation method (Treece et al., 2000; **Figure 1A**). Additionally, volume, regional CSA, and 3D centroid location of each slice of the free Achilles tendon were exported in csv format. Tendon length was calculated as the cumulative sum of the distance between the centroid of consecutive slices (Obst et al., 2014a). Average CSA of the free Achilles tendon was calculated by dividing volume by length. Free Achilles tendon geometry obtained from anatomical MRI scans was used to report free Achilles tendon geometry (volume, length, average, and regional CSA) in the trained runners and healthy controls group.

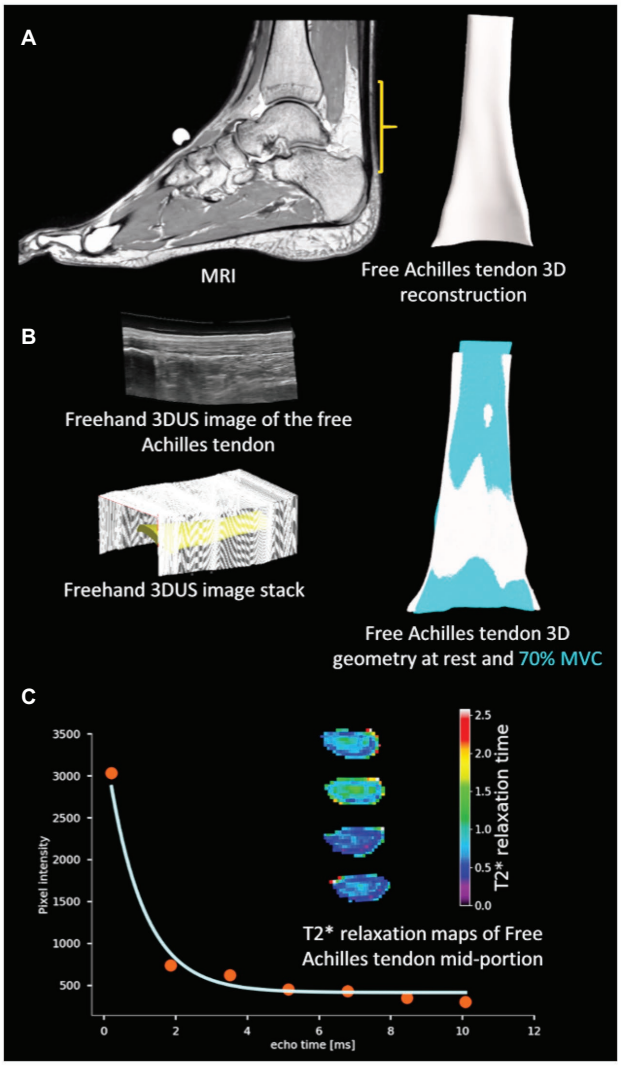

FIGURE 1 | Overview of methodology: **(A)** three-dimensional (3D) reconstruction of the free Achilles tendon from MRI **(B)** 3D ultrasound (3DUS) reconstruction of free Achilles tendon from freehand 3DUS images and free Achilles tendon geometry during rest and active muscle contraction condition [70% maximal voluntary contraction (MVC)], and **(C)** T2* relaxation time of a sample pixel in the free Achilles tendon by fitting an exponentially decaying curve, and sample colour maps of image slices of the free Achilles tendon mid-portion.

The moment arm of the free Achilles tendon was measured from the anatomical MRI scans acquired from all the participants with the ankle positioned at 0° dorsiflexion. A sparse manual segmentation of the talus was performed in the transverse plane. Sparse contour interpolation method was used to reconstruct the talus 3D surface. Following this, a cylinder was fit to the dome of the talus using in-built MATLAB functions (Torr and Zisserman, 2000) and visually inspected for correctness. The points defining the axis and centroid of the cylinder were exported and a cubic spline interpolation was performed. Similarly, a cubic spline interpolation of the centroid points of each slice of the free Achilles tendon was

also performed. The shortest distance between the two interpolated curves (i.e., axis of the cylinder and centroid line of the free Achilles tendon) was obtained using n-dimensional nearest point search method (Barber et al., 1996) and was defined as the free Achilles tendon moment arm (Alexander et al., 2017).

An open source python module (Millman and Brett, 2007; Nipy/Nibabel: https://doi.org/10.5281/zenodo.1287921) was used to parse PAR/REC files while custom MATLAB scripts were used to parse XML/REC files. $T2^*$ relaxation time of each pixel was obtained by fitting a mono-exponential decay curve with bias to the pixel values measured at different echo times using Levenberg-Marquardt algorithm implemented in open source python module (Newville et al., 2016; *lmfit*: https://doi.org/10.5281/zenodo.2620617; **Figure 1C**). In the first step, the exponential model available in *lmfit* was used to estimate the initial parameters. Following this step, a custom exponential model accounting for bias was created and the parameters estimated in the first step were used to estimate the $T2^*$ relaxation time. A manual segmentation of the free Achilles tendon cross-section was performed to extract individual $T2^*$ relaxation time values and the average $T2^*$ relaxation time from the cropped pixels was obtained for each participant.

Freehand 3DUS

All freehand 3DUS data were acquired, processed, and analysed by the same experimenter (DD) to quantify free Achilles tendon mechanical stiffness, Young's modulus, and length normalised mechanical stiffness. 2D ultrasound (Aplio 500, Canon Medical Systems Corporation, Otawara, Tochigi, Japan) and motion capture (Vicon MX T series, Vicon Motion Systems Ltd., Oxford, England, UK) data were acquired at 30 and 100 Hz, respectively. The ultrasound transducer (PLT-805AT, linear probe, width: 58 mm, frequency range: 5–12 MHz, Canon Medical Systems Corporation, Otawara, Tochigi, Japan) was fitted with six rigidly attached retroreflective markers. A pulse signal generated via an external trigger was used to synchronise 2D ultrasound and motion capture data. The ultrasound imaging parameters were kept constant at both experimental test centres (10 MHz, depth: 30 mm, resolution: 0.063 mm, capture frequency: 30 Hz, acoustic power: 100%, and mechanical index: 1.5). Prior to acquisition, temporal and spatial calibration of the ultrasound transducer was performed in a water bath (35°C) using a single wall phantom procedure (Prager et al., 1998). Visualisation of the free Achilles tendon cross-section was enhanced by securely attaching an acoustic standoff pad (~20 mm) to the transducer during data acquisition. A thin layer of ultrasound transmission gel (Other-sonic, Pharmaceutical Innovations, Newark, NJ, USA) was applied to the participant's skin to ensure smooth movement of the ultrasound transducer. Participants were positioned prone on a bed with their foot firmly secured to a foot plate with in-built dynamometer (Australian Institute of Sport: HUMAC NORM, Stoughton, MA, USA, Griffith University: Futek TFF600, Irvine, California, USA). The foot plate was locked with the ankle in a neutral position. The knee joint was fully extended and the hip joint was in neutral position (i.e., participant laid flat on the dynamometer bed).

Tendon Mechanical Testing

Free Achilles tendon mechanical stiffness, Young's modulus, and length normalised mechanical stiffness were obtained using a staged isometric plantarflexion contraction protocol (Peltonen et al., 2010). Freehand 3DUS images of the free Achilles tendon were acquired using a single transverse sweep (**Figure 1B**) at rest and three submaximal isometric loading conditions (25 ± 5, 50 ± 5, and 70 ± 5% of maximal voluntary isometric plantarflexion torque at neutral ankle angle). A minimum of three trials were obtained for each loading condition. Maximal voluntary isometric plantarflexion torque was obtained prior to tendon testing using the peak torque value from three 3–5 s ramped contractions. Visual feedback was used to ensure each target torque was reached and maintained throughout each stage. Scan duration was 15–25 s for the resting scans and 10–15 s during the submaximal contractions. Recorded data were discarded if the participant was unable to maintain the target torque within the specified range during submaximal isometric loading conditions.

All freehand 3DUS data were resliced at one-pixel resolution using Stradwin to ensure that errors due to misidentification of most proximal and distal slice of the free Achilles tendon were minimised (Devaprakash et al., 2019). Segmentation steps performed on MRI data were repeated on freehand 3DUS data. The calcaneal notch of four trained runners could not be imaged as the dynamometer foot plate heel support partially covered the participant's calcaneus. In these four instances, the most distal part of the calcaneus visible in 3DUS scan was identified and used as common starting point for the segmentation of the free Achilles tendon. The free Achilles tendon of each participant was segmented at each of the four different loading conditions. Tendon force at each condition was calculated by dividing the experimental ankle torque with the tendon moment arm obtained from the anatomical MRI. The experimental ankle torque represented the measured net ankle torque under load minus the measured net ankle torque at rest. Mechanical stiffness ($N.mm^{-1}$) of the free Achilles tendon was calculated by obtaining the slope of the line fitted to the linear region of the force-elongation data measured at 25, 50, and 70% active muscle contraction conditions. Young's modulus (GPa) of the free Achilles tendon was calculated by obtaining the slope of the line fitted to the linear region of the tendon stress-strain curve measured at 25, 50, and 70% active muscle contraction conditions. Length normalised mechanical stiffness (kN/strain) was calculated as the product of free Achilles tendon mechanical stiffness and resting length (Mersmann et al., 2017). Free Achilles tendon length calculated from freehand 3DUS data during resting scan was used as a reference measure in deformation and strain calculations. Tendon stress was calculated as tendon force divided by the average CSA of the free Achilles tendon measured during the active muscle contraction condition, and as such, represented the "true" tendon stress (Obst et al., 2014b). Tendon strain (%) was calculated as the change in length compared to resting length divided by the resting length.

Statistical Analysis

Analysis of variance (ANOVA) was used to assess the effect of group (trained runners versus healthy controls) on participant characteristics, Achilles tendon geometry, mechanical stiffness, Young's modulus (including parameters used to derive stiffness and modulus), and length normalised mechanical stiffness. A Kruskal-Wallis H-test was used to assess group differences in $T2^*$ relaxation time. All statistical analyses were performed using an open source python module (Vallat, 2018; Pingouin: https://doi.org/10.5281/zenodo.3386497). Additionally, statistical parametric mapping independent sample *t*-test (Pataky, 2012) was used to assess group differences in regional CSA measured at 1% intervals across the full length of the free Achilles tendon. Prior to conducting the statistical tests, all data were assessed for normality using Shapiro-Wilk test. Statistical parametric mapping test was conducted using an open source python module (spm1d: http://www.spm1d.org/). The relationship between $T2^*$ relaxation time and Young's modulus was reported using Pearson's product moment correlation coefficient.

RESULTS

The healthy controls were significantly older and had higher body mass index than the trained runners but were not different in height, body mass, or tibial length (**Table 1**). Volume of the free Achilles tendon was not significantly different between trained runners and healthy controls ($F_{1, 30}$ = 1.071, p = 0.31, η^2 = 0.03; **Figure 2A**). Length of the free Achilles tendon was significantly shorter in trained runners compared to healthy controls ($F_{1, 30}$ = 6.675, p = 0.01, η^2 = 0.18; **Figure 2B**). Average CSA of the free Achilles tendon was significantly larger in trained runners compared to healthy controls ($F_{1, 30}$ = 7.662, p = 0.009, η^2 = 0.20; **Figure 2C**). Regional CSA of the free Achilles tendon in the distal region to mid region was also significantly larger in trained runners (**Figure 3**). There was no significant group difference in tendon moment arm (**Table 1**, $F_{1, 30}$ = 0.578, p = 0.45, η^2 = 0.02).

Average $T2^*$ relaxation time of the free Achilles tendon was significantly longer in trained runners compared to healthy controls [$H(1)$ = 18.876, p < 0.001, η^2 = 0.59, **Figure 4A**]. Mechanical stiffness of the free Achilles tendon was significantly higher in trained runners ($F_{1, 29}$ = 10.198, p = 0.003, η^2 = 0.26, **Figure 4B**), however Young's modulus ($F_{1, 29}$ = 0.053, p = 0.71, η^2 = 0.002, **Figure 4C**) and length normalised mechanical stiffness ($F_{1, 29}$ = 1.933, p = 0.17, η^2 = 0.062, **Figure 4D**) were similar between the two groups. Maximum torque (runners: 95.1 ± 21.4 Nm, controls: 81.7 ± 19.9 Nm, $F_{1, 29}$ = 3.261, p = 0.08, η^2 = 0.101) and maximum force (runners: 1,898 ± 377 N, controls: 1,664 ± 281 N, $F_{1, 29}$ = 3.861, p = 0.06, η^2 = 0.118) recorded during tendon mechanical testing session were similar between the two groups. Torque, force, elongation, stress, and strain used to calculate mechanical stiffness and Young's modulus of the free Achilles tendon are reported in **Table 2**. Force measured during 70% maximal voluntary contraction (MVC) condition was significantly higher in trained runners when compared to the healthy control group ($F_{1, 29}$ = 4.262, p = 0.05, η^2 = 0.13, **Table 2**). Free Achilles tendon elongation measured during 70% MVC was significantly higher in the healthy control group when compared to trained runners ($F_{1, 29}$ = 5.363, p = 0.03, η^2 = 0.16, **Table 2**). During 25% MVC, free Achilles tendon stress was significantly higher in healthy controls compared to trained runners ($F_{1, 29}$ = 7.018, p = 0.01, η^2 = 0.20, **Table 2**). The Pearson's product moment correlation coefficient between $T2^*$ relaxation time and Young's modulus were −0.25 for all participants (p = 0.16), −0.37 for trained runners (p = 0.18), and −0.23 for healthy controls (p = 0.40; **Figure 5**).

TABLE 1 | Participant characteristics by group.

	Runners	Controls	F, *p*
Age (years)	25.2 ± 5.0	30.3 ± 4.9	8.6, 0.01
Height (cm)	175.5 ± 7.3	172.4 ± 10.5	0.9, 0.35
Body mass (kg)	64.4 ± 8.4	71 ± 16.8	2.0, 0.20
Body mass index (kg m^{-2})	20.9 ± 1.8	23.8 ± 4.5	5.7, 0.02
Tibial length (cm)	41.6 ± 2.2	41.3 ± 3.1	0.11, 0.74
Achilles tendon moment arm (cm)	5.0 ± 0.3	4.9 ± 0.5	0.462, 0.50

Data are mean ± one standard deviation.

DISCUSSION

This is the first study to comprehensively report geometry and mechanical properties of the free Achilles tendon of trained elite/sub-elite middle-distance runners. Consistent with our hypothesis, the free Achilles tendons of trained elite/sub-elite middle-distance runners were stiffer due to an altered geometry. We also found that $T2^*$ relaxation time was longer in trained runners, which is believed to be indicative, at least in part, of collagen disorganisation (Juras et al., 2013; Gärdin et al., 2016). The finding that $T2^*$ relaxation time was not significantly correlated with Young's modulus suggests that $T2^*$ relaxation time should not be used to infer free Achilles tendon material properties.

The average resting free Achilles tendon CSA was ~16% larger in trained middle-distance runners compared to healthy controls, which is broadly consistent with reports of a 15% higher CSA in endurance runners compared to healthy controls (Wiesinger et al., 2016), although not as high as the 30–36% larger CSA in runners compared to healthy controls and non-weight bearing athletes reported by Rosager et al. (2002) and Kongsgaard et al. (2005), respectively. We also found that the free tendon CSA of runners was significantly larger at the distal (calcaneal) end when compared with healthy controls. We also observed an increase in tendon CSA along the length of the free Achilles tendon (i.e., proximal end to distal end), in both runners and healthy controls consistent with a prior report in an asymptomatic population (Obst et al., 2014b). A significantly larger free Achilles tendon CSA compared to healthy controls likely reflects a positive adaptation for runners resulting in higher free Achilles tendon stiffness and concomitant reduction in the peak strain experienced during dynamic activities, as well as mitigation of mechanical fatigue (Buchanan and Marsh, 2001).

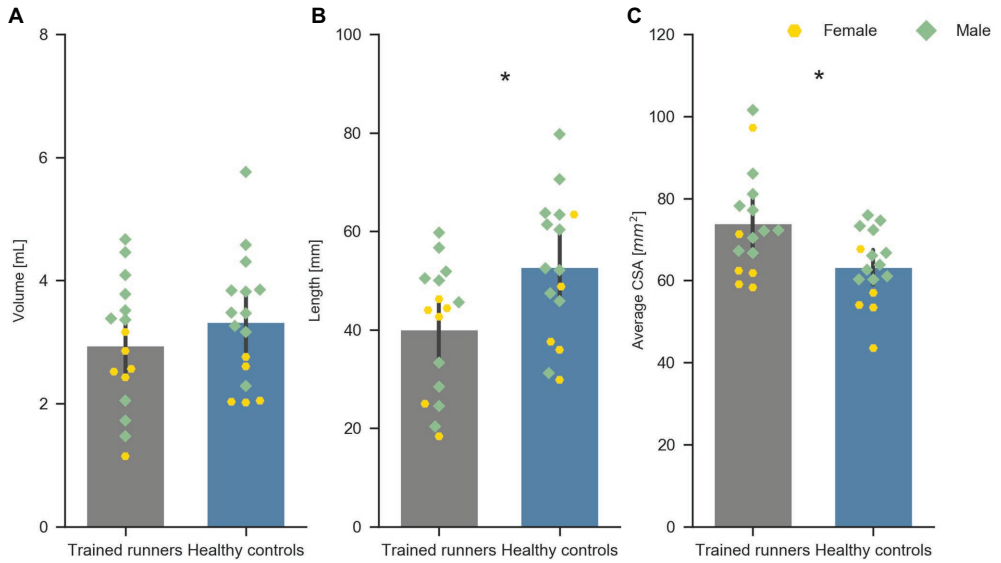

FIGURE 2 | Volume **(A)**, length **(B)**, and average CSA **(C)** of the free Achilles tendon of trained middle-distance runners (n = 16) and healthy control group (n = 16). *Indicates significant difference between the two groups.

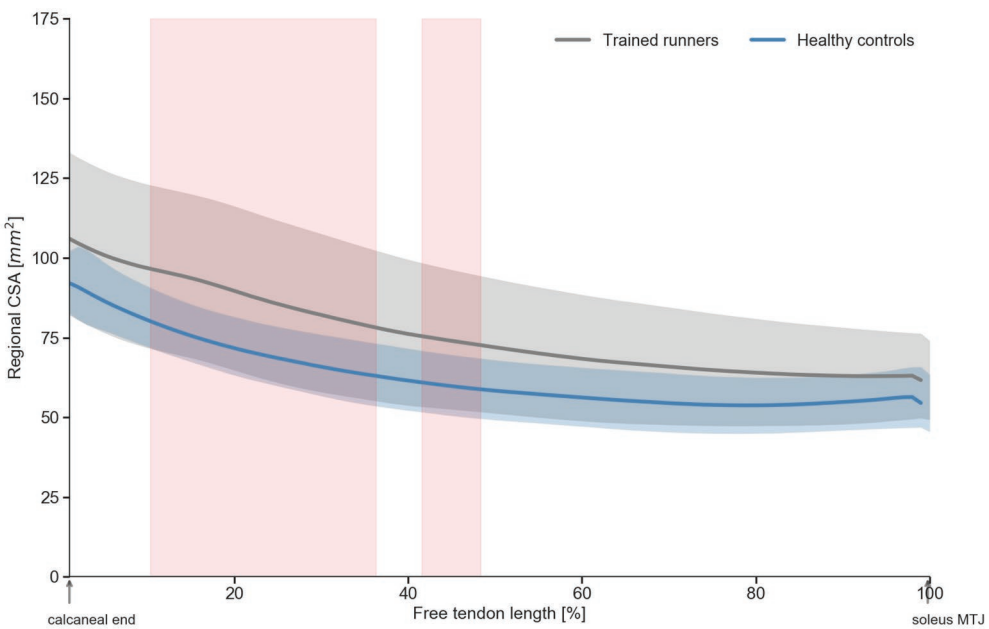

FIGURE 3 | Regional cross-sectional area (CSA) of the free Achilles tendon of trained middle-distance runners and healthy control group. Shaded regions (in red) indicate significant (p>0.05) difference in regional CSA between trained middle-distance runners and healthy control group.

The free Achilles tendon length was significantly shorter in trained middle-distance runners compared to healthy controls, and as such, we found no difference in free Achilles tendon volume between the two groups. A short and stiff tendon with larger CSA may represent a favourable adaptation for short- and middle-distance runners, as this enables the rapid transfer of greater triceps surae muscle force to the calcaneal insertion (Biewener and Roberts, 2000; Roberts, 2002). However, it is difficult to determine whether this in fact reflects an adaptation due to training or represents a preferred phenotype for middle-distance runners. In this context, it is plausible that in middle-distance runners, force production may be favoured over metabolic power consumption, as a stiffer free Achilles tendon would enable more direct transmission of muscle force (Roberts, 2002).

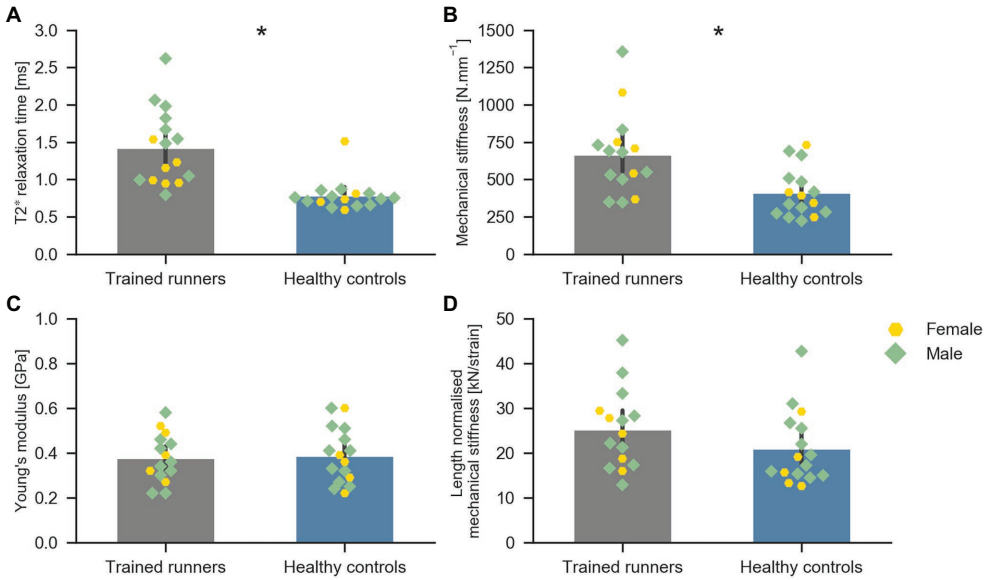

FIGURE 4 | Average T2* relaxation time of the free Achilles tendon of trained middle-distance runners (n = 16) and healthy control group (n = 16) **(A)**, Mechanical stiffness (N mm^{-1}) of the free Achilles tendon of trained middle-distance runners (n = 15) and healthy control group (n = 16) **(B)**, Young's modulus of the free Achilles tendon of trained middle-distance runners (n = 15) and healthy control group (n = 16) **(C)**, and length normalised mechanical stiffness of the free Achilles tendon of trained middle-distance runners (n = 15) and healthy control group (n = 16) **(D)**. *Indicates significant difference between the two groups.

TABLE 2 | Torque, force, elongation, stress, and strain data recorded during free Achilles tendon mechanical testing using freehand three-dimensional ultrasound.

	25% MVC		50% MVC		70% MVC	
	Runners	**Controls**	**Runners**	**Controls**	**Runners**	**Controls**
Torque (Nm)	22.8 ± 5.8	22.2 ± 5.7	47.3 ± 10.6	41.0 ± 9.5	67.0 ± 15.2	56.4 ± 14.6
Force (N)	456 ± 105	453 ± 85	943 ± 188	837 ± 131	1,337 ± 266*	1,158 ± 214
Elongation (mm)	0.77 ± 0.31	0.88 ± 0.42	1.48 ± 0.35	1.84 ± 0.63	2.07 ± 0.62	2.63 ± 0.71*
Stress (MPa)	6.38 ± 0.94	7.62 ± 1.56*	13.25 ± 2.29	14.37 ± 2.62	19.66 ± 2.57	20.22 ± 4.01
Strain (%)	1.92 ± 0.59	1.65 ± 0.67	3.82 ± 0.62	3.38 ± 0.98	5.26 ± 0.59	4.94 ± 1.08

Data are mean ± one standard deviation.
**Indicates significant difference between runners and healthy controls.*

Further, since we did not measure the whole Achilles tendon geometry (i.e., free Achilles tendon and aponeurosis), we do not know what influence a short free Achilles tendon would have on the mechanical behaviour of the whole Achilles tendon-aponeurosis. In the present study, we decided to present absolute measurements of free Achilles tendon geometry rather than normalised values as our findings did not change when normalising free Achilles tendon CSA to participant mass or free Achilles tendon length to tibial length.

The mechanical stiffness of the free Achilles tendon was 47% higher in trained runners compared to healthy controls. Our estimates of free Achilles tendon stiffness (trained runners = 667 ± 275 N.mm^{-1} and healthy controls = 410 ± 164 N.mm^{-1}) are similar to those reported for free Achilles tendon by Magnusson et al. (2003) (759 ± 132 N.mm^{-1}); however, the mechanical stiffness values we have reported are greater than the values reported by other studies (Rosager et al., 2002; Arampatzis et al., 2010; Arya and Kulig, 2010) for the whole Achilles tendon-aponeurosis system. The mechanical stiffness reported by Kongsgaard et al. (2011) for the free Achilles tendon (2,622 ± 534 N.mm^{-1}) are almost four times greater than the values reported in the present study. Our estimates of Young's modulus (trained runners = 0.38 ± 0.11 GPa and healthy controls = 0.39 ± 0.12 GPa) were close to the lower end of Young's modulus values reported by *in vivo* (0.78 ± 0.18 GPa; Magnusson et al., 2003) and *in vitro* (0.38 ± 0.10 GPa; Lewis and Shaw, 1997; 0.82 ± 0.21 GPa; Wren et al., 2001) studies of the free Achilles tendon. The wide variation in mechanical stiffness and Young's modulus values reported in the literature may be due to differences in the portion of the tissue (whole Achilles tendon-aponeurosis vs. free Achilles tendon) analysed and imaging methods (2D vs. 3D) used to track tissue deformation. For instance, cadaveric Achilles tendon samples studied by Wren et al. (2001) included a substantial portion of the Achilles tendon aponeurosis;

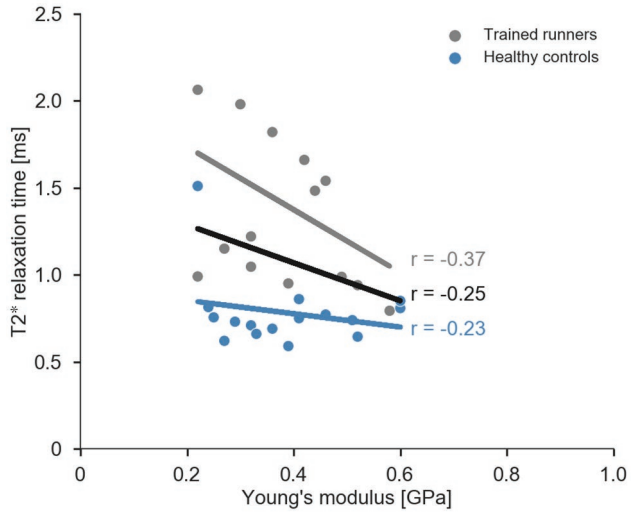

FIGURE 5 | Scatterplot and regression lines (combined data: black, trained runners: grey, and healthy controls: blue) for relationship between average T2* relaxation time and Young's modulus of the free Achilles tendon.

whereas Lewis and Shaw (1997) analysed the free Achilles tendon portion only. It is possible that Wren et al. (2001) may have under-estimated the strain experienced by the free Achilles tendon for a given load and may have over-estimated the Young's modulus of the samples tested. Nonetheless, we analysed the free Achilles tendon region similar to Lewis and Shaw (1997) and the Young's modulus values reported in the two studies are similar. Furthermore, the freehand 3DUS approach used to assess free Achilles tendon geometry (volume, length, and CSA) in the present study has been shown to provide high levels of test-retest reliability (intra-class correlation coefficient > 0.98) and accuracy of phantom length measures (standard error = 0.2 mm; Obst et al., 2014a). Consistent with other studies of the Achilles tendon, our results suggest that changes in the free Achilles tendon mechanical stiffness in trained elite/sub-elite middle-distance runners are primarily driven by alterations in free Achilles tendon geometry, rather than changes in Young's modulus (Wiesinger et al., 2015, 2016).

T2* relaxation times of the mid-portion of the free Achilles tendon were significantly longer in runners compared to non-runners. Longer T2* relaxation time in tendons has been suggested to represent greater amount of free water protons between collagen fibres that could reflect increased water content and/or disorganised collagen alignment (Diaz et al., 2012; Juras et al., 2012, 2013). Our T2* relaxation times were computed using a mono-exponential decay function, and therefore, represent the weighted mean of fast and slow T2* relaxation times (Juras et al., 2013). Therefore, we cannot say whether our results reflect increased content of bound or unbound "free" water molecules, which may be possible using a bi-exponential decay function to measure the fast and slow T2* relaxation times, respectively. Regardless, our results are broadly supported by studies that report longer mono-exponential T2* relaxation times in recreational runners versus non-runners (Grosse et al., 2015), and in individuals with tendon pathology versus healthy controls (Juras et al., 2013; Gärdin et al., 2016). Longer T2* relaxation time in runners is also consistent with findings of Hullfish et al. (2018) who reported the lower collagen alignment and ultrasound echogenicity in runners compared to non-runners. Taken together with the findings of Grosse et al. (2015) and Hullfish et al. (2018), the findings of the present study indicate that the free Achilles tendon of trained elite/sub-elite middle-distance runners exhibits features also reported for pathological tendons (i.e., increased CSA and poorly organised collagen) and appear to place runners on a continuum of Achilles tendon T2* relaxation time that is intermediate between healthy and pathological Achilles tendons. Further, T2* relaxation time has been shown to correlate with clinical scores in Achilles tendinopathy (Juras et al., 2013). The lack of significant correlation between T2* relaxation time and Young's modulus from the present study suggests that T2* relaxation time should not be considered a valid proxy for the free Achilles tendon Young's modulus. There are several possible explanations for this finding. Firstly, T2* relaxation time may primarily reflect the hydration state of the tissue, to a greater extent than the degree of collagen disorganisation, and so a greater concentration of free water in runner would not be expected to influence material properties. Alternatively, the extent of collagen disorganisation detected in runners may be below the threshold required to influence material properties of the tendon.

Limitations

There are several limitations to this study. Firstly, while all MRI scans were obtained on the same model of MRI machine and using the same MRI sequences, the two participant groups were scanned at different facilities, so we cannot fully discount the possibility of a systematic error in T2* relaxation time estimates. Nonetheless, T2* relaxation times reported in the present study are within the range of values reported in the literature (Juras et al., 2012; Grosse et al., 2015) and followed similar trends. Further, the T2* relaxation time reported in this study was the mean value obtained from pixels within the tendon CSA within five image slices (mid-portion of the free Achilles tendon). Future studies should examine regional variation in T2* relaxation time. While we performed free Achilles tendon measurements in carefully controlled conditions, it is still possible that small errors in ankle joint torque measures could be present. The triceps surae muscle forces used to compute mechanical stiffness of the free Achilles tendon were calculated as the ratio between measured ankle plantarflexion moment and moment arm measured during a static MRI. As the measured ankle plantarflexion moment is influenced by the activity of muscles other than the triceps surae (e.g., tibialis anterior), the EMG signals of the triceps surae and tibialis anterior muscles were visually inspected and instructions were provided by the operator to the participant to prevent co-contraction between the triceps surae and tibialis anterior muscles. Furthermore, we ensured that the participant's foot was firmly secured to the foot plate of dynamometer and the ankle joint axis was aligned with the dynamometer axis. However, some misalignment between the ankle joint axis and dynamometer axis could have been present during some of

the high intensity active muscle contractions (70% MVC). We also acknowledge that the two participant groups differed in age on average by 5 years and were not equally balanced by gender and that this may had a small influence on our overall findings. Finally, given the cross-sectional design of the study, it is not possible to determine whether the observed differences in free Achilles tendon properties reflect training adaptations or are to some extent pre-existing properties that favour running performance. Prospective studies are required to address this question. Modelling studies that account for an individual's free Achilles tendon properties are also required to understand the effects of training induced tendon adaptations on triceps surae fascicle behaviour and running economy.

CONCLUSION

The free Achilles tendon of trained elite/sub-elite middle-distance runners is stiffer than healthy controls primarily due to an altered geometry. In the absence of group differences in tendon material properties, the larger free Achilles tendon CSA in runners compared to healthy controls appears to be the primary mechanism that protects the free Achilles tendon of runners against high stresses and strains. Although $T2^*$ relaxation time is sufficiently sensitive to detect differences between trained runners and healthy controls, $T2^*$ relaxation time should not be considered a valid proxy for Achilles tendon material properties.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were approved by the Human Research Ethics Committee's at Griffith University and the Australian Institute of Sport. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DD, SO, DL, RB, BK, IB, NV, AH, DP, and CP conceived and designed the research. DD, CP, SJO, BK, IB, KA, TC, GD, AH, DP, and NV conducted the experiments. DD, CP, SO, RB, and DL analysed and interpreted the data. DD, SO, RB, and CP wrote the initial manuscript draft. All authors contributed to the article and approved the submitted version.

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Effects of Surface Properties on Gastrocnemius Medialis and Vastus Lateralis Fascicle Mechanics During Maximal Countermovement Jumping

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Interactions between human movement and surfaces have previously been studied to understand the influence of surface properties on the mechanics and energetics of jumping. However, little is known about the muscle-tendon unit (MTU) mechanics associated with muscle activity and leg adjustments induced by different surfaces during this movement. This study aimed to examine the effects of three surfaces with different properties (artificial turf, hybrid turf, and athletic track) on the muscle mechanics and muscle excitation of the gastrocnemius medialis (GM) and vastus lateralis (VL) during maximal countermovement jumping (CMJ). Twelve participants performed maximal CMJs on the three sport surfaces. GM and VL muscle fascicles were simultaneously imaged using two ultrafast ultrasound systems (500 Hz). MTUs lengths were determined based on anthropometric models and two-dimensional joint kinematics. Surface electromyography (EMG) was used to record GM and VL muscle activity. Surface mechanical testing revealed systematic differences in surface mechanical properties (P = 0.006, η^2: 0.26–0.32, *large*). Specifically, the highest force reduction and vertical deformation values have been observed on artificial turf (65 ± 2% and 9.0 ± 0.3 mm, respectively), while athletic track exhibited the lowest force reduction and vertical deformation values (28 ± 1% and 2.1 ± 0.1 mm, respectively) and the highest energy restitution (65 ± 1%). We observed no significant difference in CMJ performance between the three surfaces (∼35–36 cm, P = 0.66). GM and VL fascicle shortening (P = 0.90 and P = 0.94, respectively) and shortening velocity (P = 0.13 and P = 0.65, respectively) were also unaffected by the type of surface. However, when jumping from greater deformable surface, both GM muscle activity (P = 0.022, η^2 = 0.18, *large*) and peak shortening velocity of GM MTU (P = 0.042, η^2 = 0.10, *medium*) increased during the push-off phase. This resulted in a greater peak plantar

flexion velocity late in the jump (P = 0.027, η^2 = 0.13, *medium*). Our findings suggest that maximal vertical jumping tasks in humans is not affected by common sport surfaces with different mechanical properties. However, internal regulatory mechanisms exist to compensate for differences in surface properties.

Keywords: ultrasound, fascicle, surface stiffness, power amplification, electromyography, jumping

INTRODUCTION

Maximal vertical jumping performance mainly depends on the mechanical power generated by the lower limb muscle-tendon units (MTU) during the push-off phase (Bobbert et al., 1986; Anderson and Pandy, 1993; Kurokawa et al., 2003; Farris et al., 2016; Nikolaidou et al., 2017; Wade et al., 2019). As such, jumping movement has been analyzed in light of muscle and tendon behaviors (Bobbert et al., 1986; Anderson and Pandy, 1993; Kurokawa et al., 2003; Farris et al., 2016; Nikolaidou et al., 2017; Wade et al., 2019) and there is evidence for a decoupling mechanism between muscle fascicles and joint motion thanks to the compliance of the tendinous tissues (Alexander, 1974; Holt, 2019). Specifically, tendinous tissues (connective tissues: extracellular matrix, aponeurosis, tendon) can act like springs by storing elastic strain energy and rapidly releasing it to power body movements (Alexander and Bennet-Clark, 1977; Roberts, 2016). This "catapult-like" mechanism allows the amplification of MTUs' power outputs far beyond the contractile power capabilities of the muscle (Alexander and Bennet-Clark, 1977; Roberts and Azizi, 2011). These mechanical interactions between muscle and tendon are modulated by the nervous system, and the level and timing at which a muscle is activated directly influences power amplification by the tendinous tissues (Anderson and Pandy, 1993; Bobbert et al., 1996; Wade et al., 2019) as well as the direction of energy flow (e.g., from muscle to tendon to body) (Roberts and Azizi, 2011; Roberts, 2019). During jumping, a proximal-to-distal timing of leg muscle excitation patterns from the hip to the ankle was previously reported (Bobbert and van Ingen Schenau, 1988; Voigt et al., 1995). This sequence is similar to the kinematics sequence of joint extension during jumping (Gregoire et al., 1984; Bobbert and van Ingen Schenau, 1988; Voigt et al., 1995) and allows the appropriate transformation of joint rotations into translation of the center of mass upward through the coordinated action of biarticular and monoarticular muscles during push-off (Gregoire et al., 1984; Van Ingen Schenau, 1989).

During terrestrial locomotion, the surface/substrate is loaded under the body weight and can act like as an additional spring in series affecting movement efficiency (Bosco et al., 1997; Ferris and Farley, 1997; Kerdok et al., 2002), intrinsic stability (Daley and Biewener, 2006), energy dissipation (Hollville et al., 2019), and/or performance (McMahon and Greene, 1979; Arampatzis et al., 2004; Reynaga et al., 2019). However, it seems that in humans, varying common indoor and outdoor sports surfaces do not improve or impair maximal jumping and sprinting performance (Stafilidis and Arampatzis, 2007; Malisoux et al., 2017; Firminger et al., 2019; Hatfield et al., 2019). The main reason is probably due to the low contribution of these standardized sports surfaces to the total mechanical work performed by the human body during maximal motor tasks (Arampatzis et al., 2004; Stafilidis and Arampatzis, 2007). A previous study of sprinting on tracks with different degrees of stiffness reported only a minor surface compression (<1 cm) with no effect on sprint performance and leg mechanics (Stafilidis and Arampatzis, 2007). However, internal regulatory mechanisms may be used to maintain similar movement performance with respect to surface characteristics, or the movement could be compromised (e.g., changes in neural and joint coordination can disrupt elastic energy storage and thus affect the tuning of muscle and tendon mechanics; Sawicki et al., 2015; Reynaga et al., 2019; Roberts, 2019).

Humans adjust the way they move depending on the mechanical behavior of the surface (Arampatzis et al., 2004; Stafilidis and Arampatzis, 2007). Surface mechanical behavior is fixed and determined by surface material properties dependent on surface construction (Stafilidis and Arampatzis, 2007; Firminger et al., 2019). While classical body and joint dynamics analyses could not be sufficient to explore such adjustments, they might be detected from neuromuscular and MTU mechanics measures (Hollville et al., 2019). Indeed, we recently observed that surface absorbing capacity can affect muscle-tendon interactions during landing (Hollville et al., 2019). To our knowledge, no study has yet considered these aspects when studying the relation between external environment like sport surfaces and maximal jumping tasks.

The aim of this study was to evaluate the effects of three common sport surfaces (artificial turf, hybrid turf, and athletic track) with different mechanical properties on the fascicle mechanics and muscle excitation level of the gastrocnemius medialis (GM) and vastus lateralis (VL) muscles during maximal vertical jump. We hypothesized that (i) the influence of surface properties would be marginal for jumping performance, and (ii) surface properties would induce changes to jumping neuromechanics via adjustments in fascicle length changes and/or muscle excitation patterns without altering the proximal-to-distal joint sequence.

MATERIALS AND METHODS

Participants

Sixteen active males initially participated in this study after giving written informed consent. Due to issues identified post-data collection (for details, see section "Data Reduction and Statistical Analysis" below), four participants were excluded and our final sample was composed of 12 active males (age: 24.2 ± 2.0 years; height: 178.5 ± 6.4 cm; body mass: 72.7 ± 7.1 kg).

The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee (Ouest IV, agreement no. 16/18).

Experimental Protocol

Data collected for this study is part of a broader protocol comprising other motor tasks analyzed over the same three surfaces (Hollville et al., 2019). We chose to split the data collected in two parts regarding the different hypotheses tested and the amount of information available. We randomized motor tasks as well as the surfaces tested. Experimental protocol was carried out outdoors over three surfaces with different properties (**Figure 1A**): a third-generation artificial turf (∼40 mm pile height, sand and rubber granules combined as infill, 15 mm shock pad), a hybrid turf (substrate made of cork, sand and micro-synthetic fibers, AirFibr®, Natural Grass, Paris, France), and an athletic track (polyurethane). Participants were not specifically familiarized with one surface or another. They warmed up by running 10 min at a self-selected pace and doing dynamic stretching. Then, we placed the ultrasound transducers, EMG electrodes, reflective markers, and insole sensors on the right leg and shoe of each participant. A rapid familiarization (∼20-minute in total) was performed with the realization of maximal countermovement jumps on each surface. We ensured that CMJ performance reached a plateau (i.e., no further increase in jumping height from trial to trial) during the familiarization by providing feedback about the jumping technique (jumping with arms restricted, trunk and legs fully extended during flight time until ground contact) and flight distance (i.e., maximal height reached in the air). In the meantime, ultrasound images, EMG, and insole sensor signals were checked. In total, all participants performed approximately five countermovement jump (CMJ) on each surface before data collection. Afterward, participants performed three maximal vertical CMJ without arm swing at preferred depths over the three surfaces in a random order while data were collected. Between maximal jumps, participants had a passive rest of ∼90 s while they had ∼10 min between surface conditions, corresponding to the time needed to move the entire setup from one surface to another. All participants were familiar with performing maximal jumping tasks on the surfaces used in the present study, which are commonly used in sport practice. We standardized the shoe model so that all participants wore the same pair of molded football cleats (Adidas X16.FG, Herzogenaurach, Germany).

Surface Testing

Standardized mechanical tests (i.e., vertical impact tests) were performed by an independent surface testing institution (Novarea, Gellainville, France) to characterize surface behavior under specific loading (Hollville et al., 2019). Vertical deformation, force reduction, and energy restitution of each surface were computed from acceleration-time signals (1000 Hz; Advanced Artificial Athlete device, AAA; Deltec Equipment, Duiven, Netherlands). Briefly, force reduction represents the ability of a surface to reduce an impact load (i.e., 20 kg mass dropped from 55 mm onto a 2000 N/mm stiffness spring linked to a 70-mm diameter spherical plate) and was computed according to the following equation:

$$FR = \left[1 - \left(\frac{F_{max}}{F_{concrete}}\right)\right] \times 100 \quad (1)$$

where FR corresponds to force reduction (in %), F_{max} is the peak force obtained during the impact test, and $F_{concrete}$ is a theoretical reference force value for a concrete floor (6760 N). F_{max} was computed using the following equation:

$$F_{max} = m \times g \times G_{max} + m \times g \quad (2)$$

where G_{max} is the vertical peak acceleration during impact (g), m is the mass (i.e., 20 kg) and g is the gravitational acceleration (i.e., 9.81 $m.s^{-2}$). Vertical deformation (in mm) is the deformation of the surface under the same applied load in the vertical axis. This was calculated from the time when the spherical plate first contacts the surface until the time of the maximum absolute velocity of the mass using the equation:

$$VD = D_{max} - D_{spring} \quad (3)$$

where D_{max} is the displacement of the falling mass and D_{spring} is the displacement of the spring (Colino et al., 2017). Finally, energy restitution (in %) is determined by the energy input minus the amount of energy that has been lost in the surface. The area under the unloading force-deformation curve obtained from acceleration-signals describes the energy return of the surface (Baroud et al., 1999). This value represents the surface ability to return energy after being deformed with 100% corresponding to zero energy loss (i.e., no hysteresis). Three trials per test per surface were averaged. The experiments were performed over 3 weeks with similar forecast conditions. Specifically, the experiments took place in Paris in June in the shade (under tents) with relatively high air temperature (20–34°C). Surface maintenance (substrate decompaction, water spreading, mowing) and surface hardness homogeneity control were performed before and after each protocol, resulting in similar surface conditions between subjects.

Joint Kinematics

Since it was not possible to use a laboratory motion capture system outdoors, a high-speed video camera (300 $frame.s^{-1}$; Casio Exilim EX-F1, Japan) was used to record the two-dimensional (2D) positions of six reflective markers placed on the right side of the participants at the following locations: the fifth metatarsal, lateral calcaneus, lateral malleolus, lateral femoral epicondyle, great trochanter, and the acromion. The camera axis was perpendicular to the sagittal plane of jumping to prevent from image distortion and inaccurate marker trajectories (Kurokawa et al., 2003; Hickox et al., 2016). Marker trajectories were semi-automatically digitized (Dartfish ProSuite 9.0, Fribourg, Switzerland), low-pass filtered (8 Hz) (Kurokawa et al., 2003) and used to retrieve marker coordinates. Ankle, knee, and hip joint angles and velocity were computed at each time frame during the CMJ, and we identified the

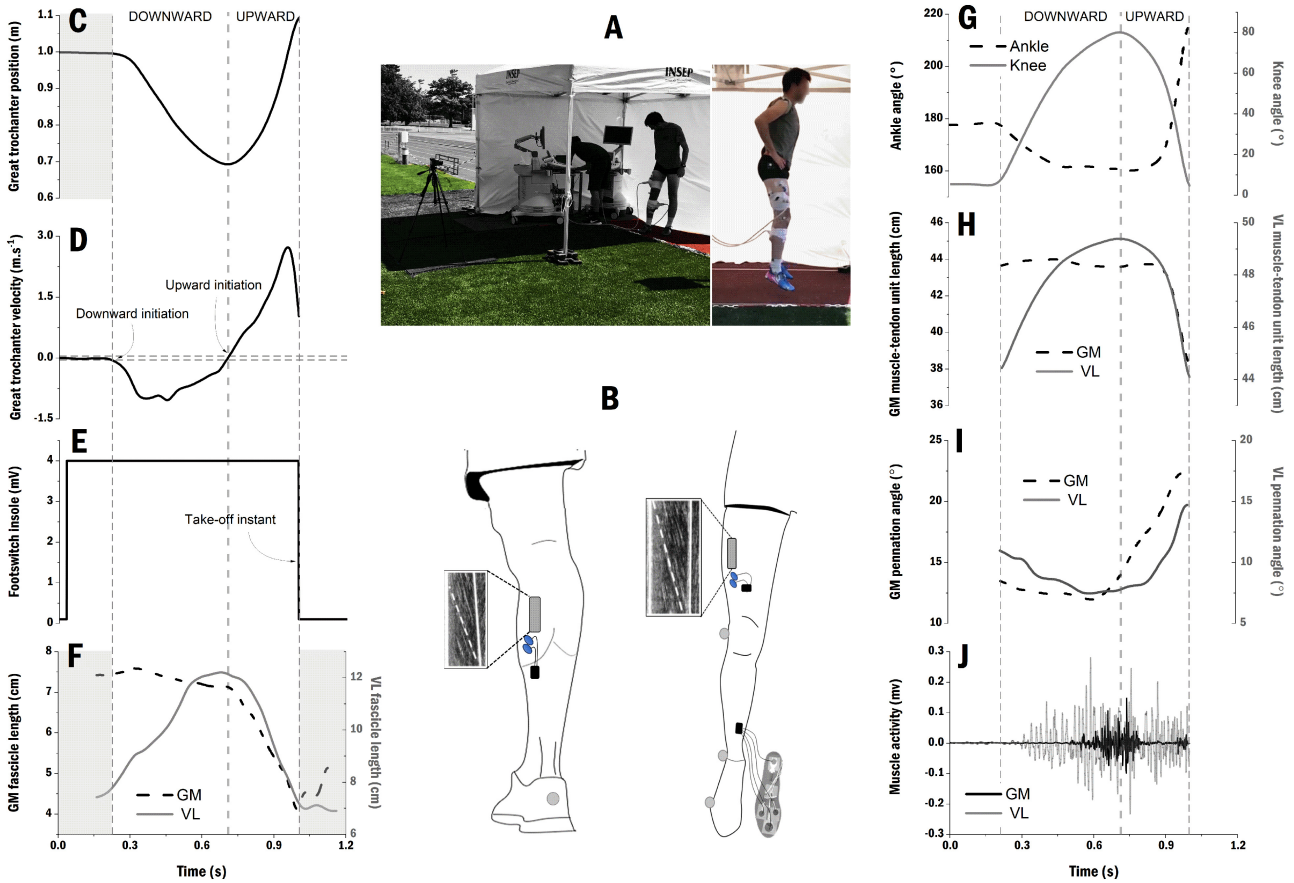

FIGURE 1 | Representative data of one participant and processing overview. **(A)** Experimental setup outdoor under a tent which was moved along the protocol over the three surfaces (i.e., hybrid turf, synthetic turf, and athletic track); **(B)** Positions of the probes and electrodes over the *gastrocnemius medialis* (GM) and *vastus lateralis* (VL) muscle bellies, and the four force resistor sensors which were placed on the insole of the right shoe to detect take-off instant; Great trochanter position **(C)** and velocity **(D)** with downward and upward initiation instants; **(E)** Force resistor sensor pattern with 4 mV corresponding to a contact between the foot and the ground and 0 mv corresponding to flight period; **(F)** GM fascicle length (dash-black line) and VL fascicle length (gray line). The shaded areas correspond to the delimitation of countermovement jump; **(G)** Ankle (dash-black line) and knee (gray line) joint angles; **(H)** GM (dash-black line) and VL (gray line) muscle-tendon units length computed over the countermovement jumps; **(I)** GM (dash-black line) and VL (gray line) pennation angle; **(J)** Raw muscle activity of GM (black) and VL (gray) muscles before processing and normalization procedure (see section "Materials and Methods").

onset of joint rotation to investigate the proximal-to-distal joint sequence (Winter, 2009). Four force sensitive resistors (Footswitch FSR sensor, Zerowire, Cometa systems, Milan, Italy; **Figures 1B,E**) were fixed onto the insole of the right shoe to synchronize jumping take-off with ultrasound data (**Figure 1E**) and to compute jump height based on flight time (Bosco et al., 1983). The flight time method estimates the flight distance during jumping and does not account for center of mass displacement before take-off. For sake of clarity, we will use the term jump height throughout the manuscript to express the maximum height reached by the center of mass (i.e., flight distance). Simultaneous take-off of both legs was visually and blindly checked by the same investigator. Kinematics data were synchronized at take-off based on the fifth metatarsal y-coordinate. The onset of downward motion was identified as when the velocity of the marker attached over the great trochanter was inferior to -0.05 m.s^{-1} (**Figure 1D**). The onset of push-off phase was identified as when the velocity of the same marker exceeded 0.05 m.s^{-1} (**Figure 1D**; Farris et al., 2016).

Muscle-Tendon Unit Mechanics

GM and VL muscle fascicles were simultaneously imaged using two ultrafast ultrasound systems (Aixplorer, Supersonic Imagine, Aix-en-Provence, France) synchronized with a common trigger to a digital converter (DT 9804, Data Translation, Marlboro, MA, United States). Two linear probes (4–15 MHz, SuperLinear 15–4, 55 mm field of view, Vermon, Tours, France) were first placed transversally over GM and VL muscle bellies to identify muscle areas; then the probe was gradually rotated longitudinally so that the probe was aligned with fascicle orientation (**Figure 1B**), then fastened using custom-made supports, elastic bandages, and tape. Participants were asked to perform low-intensity knee flexions, extensions, plantar flexions, and dorsiflexions in order to ensure that the observable part of the fascicles was

clearly visible and that the probe was oriented according to the fascicle's line of action over the entire movement. The acquisition was performed with the research mode with the following parameters: 500 Hz sample frequency, images acquired over a 2 seconds period of time, gain 30–55 dB, 8–10 MHz of ultrasound frequency. Muscle fascicles and aponeuroses length were tracked on B-mode images using a semi-automatic tracking algorithm previously validated (Cronin et al., 2011; Gillett et al., 2013; Farris and Lichtwark, 2016). For each participant, the same fascicle was identified on the initial image of all trials in order to improve reliability of fascicle length measurements between trials. Fascicle length extrapolations are inherent to fascicle dynamics tracking with single short probe field of view (Kurokawa et al., 2003; Brennan et al., 2017). Trigonometry equations were used when necessary to extrapolate GM and VL fascicle lengths (Kurokawa et al., 2003), which were then reported in both absolute (cm) and relative values (i.e., divided by fascicle length measured during a static trial where participants were standing up). Pennation angle was defined as the angle formed at the intersection between fascicle and deep aponeurosis. For both muscles, the lowest and highest pennation angle was identified and we calculated changes in pennation angle as the difference between the two values. In addition, only for GM muscle, we reported an average pennation angle between 80 and 85% of the CMJ. This latter value corresponds to the pennation angle at the time where the GM tendinous tissue length reached its maximum and GM muscle activity is declining (i.e., end of tendinous tissues energy storage – start of energy release; Kurokawa et al., 2003). MTUs' lengths were computed at each time point based on joint angles and anthropometric models (Grieve et al., 1978; Visser et al., 1990). MTU and fascicle velocity were derived from their measured lengths. Peak MTU shortening velocity and peak and average muscle fascicle shortening velocity were computed. Furthermore, peak MTU shortening velocity was divided by peak muscle fascicle shortening velocity to obtain MTU gearing. This ratio represents the amplification of MTU velocity owing to tendinous tissues compliance and fascicle rotation (or the product of the belly gearing and the tendon gearing; Wakeling et al., 2011).

Surface Electromyography

Surface electromyography (EMG) was recorded to measure GM and VL muscle activity using a wireless system (ZeroWire, Aurion, Italy), which was synchronized with the force resistive sensors and the two ultrasounds via an external trigger. Bipolar electrodes were placed longitudinally with respect to the fascicle's alignment and the ultrasound position (**Figure 1B**). Raw EMG signals were pre-amplified (input impedance; 20 MM, common mode rejection ratio: 90 dB; signal-to-noise ratio: >50 dB; gain: 1000), digitized at 2000 Hz, and then transmitted wirelessly to a remote unit. Raw EMG data were processed with a custom Matlab script (The MathWorks, Natick, MA, United States). The DC offset was removed from raw signals, then bandpass filtered (10–450 Hz), rectified, and averaged with a rolling root mean squared calculation over consecutive windows of 50 ms. To estimate muscle activity during the countermovement jump, EMG RMS data were averaged in two phases: during the last 100 ms of the countermovement (i.e., downward phase), and during the push-off phase. EMG RMS data were normalized to the averaged EMG RMS values obtained on the stiff athletic track surface in both phases (Moritz et al., 2004).

Data Reduction and Statistical Analysis

Test-retest repeatability of the maximal CMJ task between trials (data pooled between surface conditions; CV: 3.4%; SEM: 1.84 cm; ICC: 0.977) and for each surface condition (artificial turf, CV: 3.6%; SEM: 1.68 cm; ICC: 0.970; hybrid turf, CV: 4.2%; SEM: 1.92 cm; ICC: 0.980; athletic track, CV: 2.4%; SEM: 1.91 cm; ICC: 0.980) demonstrated good to very good repeatability within-subject on all surfaces. The trial resulting in the highest jump for each surface was used for statistical comparisons. As previously mentioned, due to issues with ultrasound images, four participants among the sixteen initial participants were excluded, resulting in $N = 12$ for fascicle-related data and joint kinematics. These issues were mainly large extrapolation of the fascicle, fascicle/aponeuroses curvature, or out-of-plane images. In addition, due to the challenging aspect of fixing a transducer and EMG electrodes near the same location over the muscle belly, we have prioritized placement of the transducer at the expense of an optimal placement of the electrodes. After EMG signal frequency domain analysis (Fast-Fourier Transform), filtering, and careful visual inspection of the signals, EMG data of two and four participants were faulty for GM and VL, respectively, and were therefore excluded, resulting in $N = 10$ for GM muscle activity and $N = 8$ for VL muscle activity.

All variables were analyzed from the onset of downward motion initiation until the point of take-off (**Figure 1**). Statistical analysis was performed using Origin software (Origin Pro 2018, OriginLab Corporation, Northampton, MA, United States). The effects of surface properties on MTU mechanics (i.e., GM and VL MTUs and fascicle length changes, pennation angle, average and peak shortening velocity and, MTU gearing) and joint kinematics (i.e., range of motion, angular velocity, timing of joint extension) were tested via one-way repeated-measures ANOVA. Kruskal-Wallis non-parametric tests with multiple comparisons were completed to determine the effect of type of surface on muscle excitation (EMG RMS amplitude). Non-parametric tests were also performed on surface mechanical parameters (force reduction, vertical deformation, energy restitution). For ANOVAs, a Greenhouse-Geisser correction was performed when sphericity was violated. Bonferroni *post hoc* tests were used when the results were statistically significant (i.e., $P \leq 0.05$). All grouped data are presented as means ± standard deviations (SD) and confidence intervals (CI 95%). We calculated partial eta-squared (η^2) as a measure of the effect size for significant results with $\eta^2 < 0.06$ considered as a small effect size, $0.06 < \eta^2 < 0.14$ a medium effect, and $\eta^2 > 0.14$ a large effect.

RESULTS

Mechanical testing revealed systematic differences in the resulting mechanical characteristics between the three surfaces

TABLE 1 | Surface mechanical properties and kinematics data during maximal countermovement jumping over artificial turf, hybrid turf and athletic track.

Parameters	Artificial turf	Hybrid turf	Athletic track	Statistics	
	Mean ± SD [CI 95%]	Mean ± SD [CI 95%]	Mean ± SD [CI 95%]	*P*-values	η^2
Surface mechanical behavior					
Force reduction (%)	65 ± 2 [62.7; 67.3]∗#	55 ± 2 [52.7; 57.3]∗	28 ± 1 [25.7; 30.3]	**0.006**	*0.26*
Vertical deformation (cm)	9.0 ± 0.3 [8.7; 9.3]∗#	6.3 ± 0.5 [5.7; 6.9]∗	2.1 ± 0.1 [2.0; 2.2]	**0.006**	*0.32*
Energy restitution (%)	38 ± 2 [35.7; 40.3]∗#	29 ± 2 [26.7; 31.3]∗	65 ± 1 [63.9; 66.1]	**0.006**	*0.32*
Kinematics					
Jump height (cm)	35.7 ± 5.8 [32.0; 39.4]	35.3 ± 6.6 [31.1; 39.5]	35.9 ± 6.6 [31.7; 40.1]	0.66	
Downward phase duration (s)	420 ± 53 [383; 450]	418 ± 49 [384; 446]	432 ± 48 [395; 471]	0.52	
Push-off phase duration (s)	251 ± 37 [227; 274]	246 ± 37 [222; 269]	259 ± 45 [230; 288]	0.23	
Ankle dorsiflexion (downward, in °)	23 ± 4 [20; 25]	23 ± 3 [21; 24]	22 ± 4 [19; 25]	0.80	
Knee flexion (downward, in °)	72 ± 11 [65; 79]	68 ± 12 [61; 76]	71 ± 13 [63; 80]	0.25	
Hip flexion (downward, in °)	87 ± 14 [78; 96]	87 ± 14 [78; 96]	87 ± 13 [79; 96]	0.97	
Ankle plantar flexion (push-off, in °)	56 ± 6 [52; 60]	55 ± 5 [52; 59]	56 ± 7 [51; 60]	0.88	
Knee extension (push-off, in °)	74 ± 10 [68; 80]	73 ± 10 [67; 80]	75 ± 12 [67; 83]	0.61	
Hip extension (push-off, in °)	83 ± 11 [76; 90]	84 ± 10 [78; 90]	84 ± 10 [77; 90]	0.87	
Ankle peak extension velocity (in °/s)	641 ± 51 [608; 673]	646 ± 53 [613; 679]∗	603 ± 53 [569; 636]	**0.027**	*0.13*
Knee peak extension velocity (in °/s)	656 ± 70 [612; 701]	677 ± 73 [630; 723]	658 ± 69 [615; 702]	0.31	
Hip peak extension velocity (in °/s)	526 ± 49 [495; 557]	528 ± 46 [499; 557]	524 ± 44 [496; 551]	0.87	
Joint extension sequence					
Hip extension corresponds to 0% of the push-off phase					
Knee (% of the push-off phase)	16 ± 5 [13; 19]	13 ± 3 [11; 15]	18 ± 5 [15; 21]	0.47	
Ankle (% of the push-off phase)	33 ± 9 [27; 39]	35 ± 7 [31; 39]	30 ± 10 [24; 36]	0.33	

∗Significantly different from the athletic track. #Significantly different from the hybrid turf. Values are presented as mean ± SD [confidence interval CI 95%]. Statistical significance was set at P < 0.05. Partial eta-squared η^2 is a measure of the effect size with η^2 < 0.06 considered as a small effect size, 0.06 < η^2 < 0.14 a medium effect, and η^2 > 0.14 a large effect.

(all *P* values = 0.006, η^2: 0.26–0.32, *large*; **Table 1**). The highest force reduction and vertical deformation values have been observed on artificial turf (65 ± 2% [CI: 62.7; 67.3], 9.0 ± 0.3 mm [CI: 8.7; 9.3]), while athletic track exhibited the lowest force reduction and vertical deformation values (28 ± 1% [CI: 25.7; 30.3], 2.1 ± 0.1 mm [CI: 2.0; 2.2]) and the highest energy restitution (65 ± 1% [CI: 63.9; 66.1]).

The CMJ performance was not significantly different between surfaces (*P* = 0.66) with maximal jump height ranging between 0.35 and 0.36 m (**Table 1**). Similar jump height was also associated with similar downward (*P* = 0.52) and push-off phase durations (*P* = 0.23). We observed a proximal-to-distal joint sequence with hip extension initiating the push-off, followed by knee extension (at ∼15% of the push-off phase) and ankle plantar flexion (at ∼33%; **Table 1**). This joint sequence was not influenced by the type of surface, as revealed by a constant timing of joint extension across joints (*P* values ranged from 0.33 to 0.69; **Table 1**). Joint range of flexion during the downward phase (ankle: *P* = 0.80; knee: *P* = 0.25; hip: *P* = 0.97) as well as joint range of extension during the push-off phase (ankle: *P* = 0.88; knee: *P* = 0.61; hip: *P* = 0.87) were not affected by the type of surface. We also observed a significant difference in ankle peak angular velocity during the push-off phase (*P* = 0.027, η^2 = 0.13, *medium*). Specifically, the hybrid turf exhibited a significant higher ankle plantar flexion velocity compared to the athletic track (7 ± 9%; *P* = 0.041) while we observed a trend for an increase on the artificial turf compared to the athletic track (6 ± 8%; *P* = 0.085) (**Table 1**). No difference between surfaces was observed for peak angular velocity at the knee and hip joints (*P* = 0.31 and *P* = 0.87, respectively).

The patterns of GM and VL MTU and fascicle length are depicted in **Figure 2**. During the downward phase, GM fascicles operated near-isometrically before starting to actively shorten (on average: −2.5 ± 0.6 cm; *P* = 0.90) at the end of the downward motion (**Figure 2C**). GM pennation angle was the lowest at ∼40 to 45% at the onset of GM EMG activity rise and when fascicle begins to shorten. No difference in minimum value of pennation angle was found between surfaces (*P* = 0.74, **Figure 2G** and **Table 2**). Similarly, for VL, pennation angle reached a minimum value around the transition between countermovement and push-off phase with no surface effect (*P* = 0.78, **Figure 2H** and **Table 2**). GM muscle activity was significantly different between surfaces during the last 100 ms of the downward motion, with higher EMG RMS amplitude on the artificial turf than on the athletic track (*P* = 0.047, η^2 = 0.16, *large*; **Figure 2A**). During the downward phase, GM MTU length remained almost constant (**Figure 2E**) while VL MTU lengthened without surface effect (*P* = 0.47; **Figure 2F** and **Table 2**). This lengthening was mainly driven by active fascicle lengthening (**Figure 2D**) with similar lengthening amplitude (*P* = 0.77) between surfaces (**Table 2**).

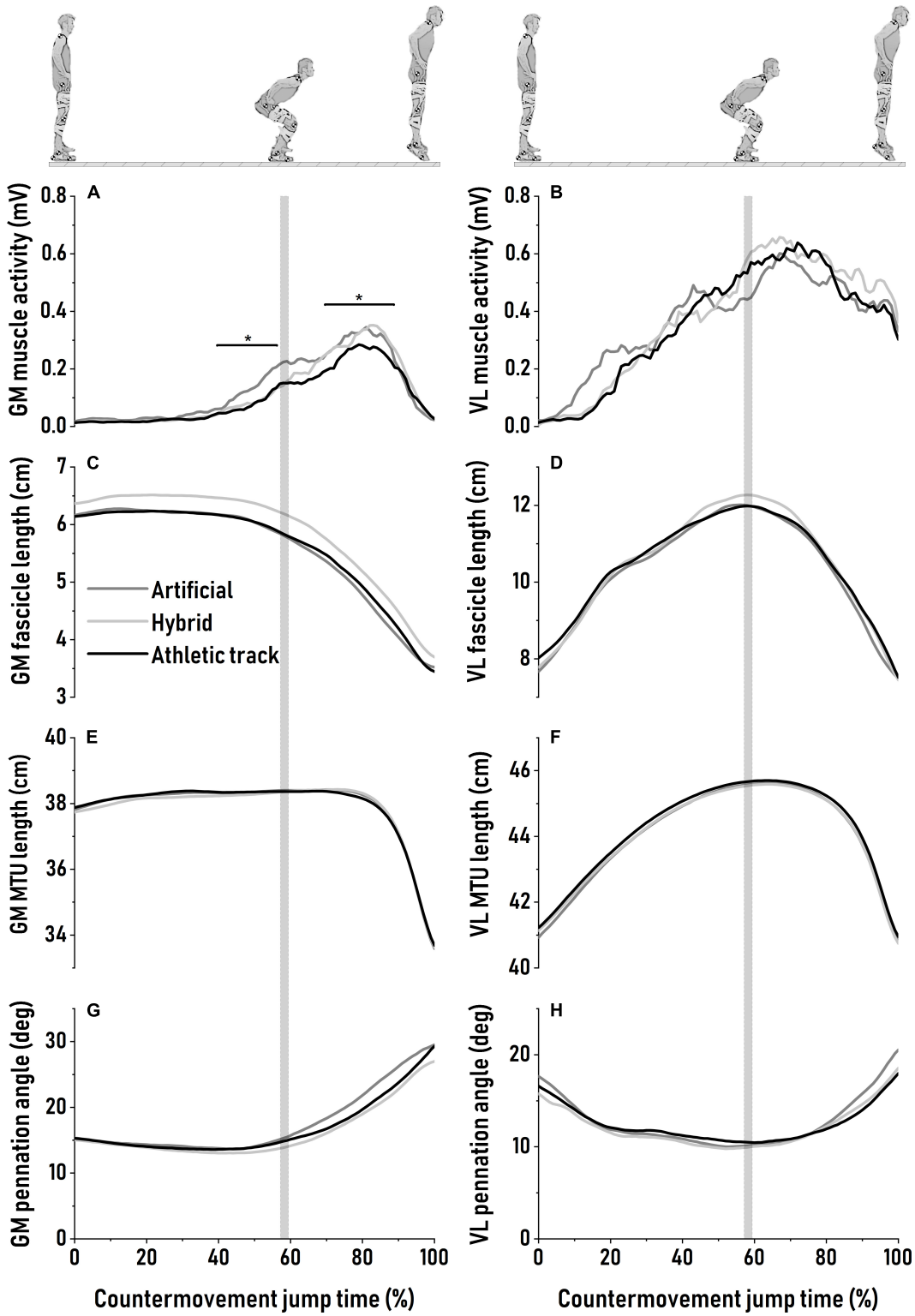

FIGURE 2 | Averaged patterns of *gastrocnemius medialis* muscle activity **(A)**, fascicle **(C)**, muscle-tendon unit **(E)** length changes and pennation angle **(G)** during maximal countermovement jumps on artificial turf (dark gray), hybrid turf (light gray) and athletic track (black). Averaged patterns of *vastus lateralis* muscle activity **(B)**, fascicle **(D)**, muscle-tendon unit **(F)** length changes and pennation angle **(H)** during the same movement over the same surfaces. Standard deviations are omitted for clarity. The vertical shaded area represents the average turning point between the end of the downward phase and the beginning of the push-off phase. Significant bars (*) indicate a significant difference from athletic track.

TABLE 2 | Muscle-tendon related-variables (n = 12) and muscle excitation amplitude (n = 10 in gastrocnemius medialis and 8 in vastus lateralis) during maximal countermovement jumping over three surfaces (artificial turf, hybrid turf and athletic track).

Parameters	Artificial turf	Hybrid turf	Athletic track	Statistics	
	Mean ± SD [CI 95%]	Mean ± SD [CI 95%]	Mean ± SD [CI 95%]	*P*-values	η^2
Gastrocnemius medialis					
Muscle-tendon unit behavior					
Shortening amplitude (cm)	−4.99 ± 0.88 [−5.55; −4.43]	−4.88 ± 0.67 [−5.31; −4.45]	−4.87 ± 0.74 [−5.43; −4.40]	0.68	
Peak shortening velocity (cm.s^{-1})	−64.1 ± 8.4 [−69.4; −58.7]*	−63.9 ± 6.1 [−67.7; −60.0]*	−59.0 ± 7.1 [−63.5; −54.4]	**0.042**	*0.10*
Fascicle behavior					
Shortening amplitude (cm)	−2.51 ± 0.58 [−2.88; −2.14]	−2.55 ± 0.64 [2.95; −2.14]	−2.49 ± 0.68 [−2.92; −2.06]	0.90	
Shortening amplitude (L/Lstanding)	−0.40 ± 0.11 [−0.47; −0.33]	−0.39 ± 0.11 [−0.46; −0.33]	−0.39 ± 0.09 [−0.45; −0.34]	0.84	
Peak shortening velocity (cm.s^{-1})	−16.1 ± 5.0 [−19.3; −12.9]	−18.9 ± 7.8 [−23.8; −13.9]	−18.2 ± 6.9 [−22.5; −13.8]	0.13	
Mean shortening velocity (cm.s^{-1})	−8.3 ± 2.3 [−9.8; −6.9]	−9.4 ± 2.5 [−11.0; −7.8]	−8.9 ± 3.0 [−10.8; −7.1]	0.16	
Muscle-tendon unit gearing	4.4 ± 1.5 [3.4; 5.3]	3.9 ± 1.6 [2.9; 4.9]	3.7 ± 1.5 [2.8; 4.6]	0.20	
Pennation angle (lowest value, deg)	13.3 ± 1.8 [11.8; 14.7]	12.8 ± 2.2 [11.4; 14.3]	13.2 ± 3.0 [11.8; 14.7]	0.74	
Pennation angle (highest value, deg)	29.8 ± 6.0 [26.7; 33.0]	27.3 ± 5.2 [24.1; 30.3]	29.5 ± 4.5 [26.3; 32.7]	0.17	
Changes in pennation angle (deg)	16.6 ± 6.0 [13.2; 19.9]	14.4 ± 5.5 [11.1; 17.8]	16.3 ± 4.6 [12.9; 19.6]	0.09	
Pennation angle at 80–85% CMJ	22.5 ± 4.4 [20.7; 24.4]	19.5 ± 2.0 [17.6; 21.4]	20.3 ± 2.8 [18.4; 22.2]	0.09	
Vastus lateralis					
Muscle-tendon unit behavior					
Lengthening amplitude (cm)	4.71 ± 0.63 [4.31; 5.10]	4.59 ± 0.70 [4.12; 5.03]	4.58 ± 0.68 [4.15; 5.02]	0.47	
Shortening amplitude (cm)	−4.82 ± 0.47 [−5.12; −4.52]	−4.85 ± 0.60 [−5.23; −4.48]	−4.76 ± 0.46 [−5.06; −4.47]	0.62	
Peak shortening velocity (cm.s^{-1})	−52.0 ± 5.6 [−55.6; −48.5]	−53.8 ± 6.4 [−57.9; −49.7]	−51.6 ± 5.0 [−54.8; −48.4]	0.27	
Fascicle behavior					
Lengthening amplitude (cm)	4.16 ± 1.28 [3.35; 4.98]	4.42 ± 1.43 [3.50; 5.33]	4.08 ± 1.98 [2.83; 5.34]	0.77	
Lengthening amplitude (L/Lstanding)	0.53 ± 0.16 [0.43; 0.63]	0.53 ± 0.13 [0.45; 0.62]	0.47 ± 0.17 [0.36; 0.58]	0.34	
Shortening amplitude (cm)	−4.47 ± 1.29 [−5.29; −3.65]	−4.60 ± 1.29 [−5.23; −3.69]	−4.33 ± 1.62 [−5.36; −3.30]	0.94	
Shortening amplitude (L/Lstanding)	−0.57 ± 0.17 [−0.68; −0.46]	−0.56 ± 0.14 [−0.65; −0.47]	−0.51 ± 0.16 [−0.61; −0.41]	0.43	
Peak shortening velocity (cm.s^{-1})	−37.0 ± 15.8 [−47.6; −26.4]	−33.7 ± 10.6 [−40.8; −26.5]	−35.7 ± 17.5 [−47.5; −24.0]	0.65	
Mean shortening velocity (cm.s^{-1})	−16.8 ± 5.6 [−20.4; −13.3]	−18.3 ± 6.8 [−22.6; −14.0]	−15.7 ± 7.1 [−20.2; −11.2]	0.39	
Muscle-tendon unit gearing	1.7 ± 0.6 [1.3; 2.1]	1.8 ± 0.8 [1.3; 2.3]	1.8 ± 0.9 [1.2; 2.4]	0.78	
Pennation angle (lowest value, deg)	9.8 ± 2.0 [8.3; 11.2]	9.4 ± 2.6 [8.0; 10.9]	10.1 ± 2.9 [8.6; 11.5]	0.78	
Pennation angle (highest value, deg)	20.8 ± 5.1 [17.6; 23.9]	19.0 ± 4.9 [15.9; 22.1]	19.2 ± 5.1 [16.0; 22.3]	0.15	
Changes in pennation angle (deg)	11.0 ± 4.8 [8.2; 13.8]	9.6 ± 5.0 [6.8; 12.4]	9.1 ± 3.8 [6.3; 11.9]	0.14	
Myoelectrical activity					
Gastrocnemius medialis (%; downward phase)	155 ± 59 [114; 196]*	122 ± 55 [84; 160]	100 ± 0	**0.047**	*0.16*
Vastus lateralis (%; downward phase)	127 ± 40 [96; 158]	103 ± 22 [86; 120]	100 ± 0	0.31	
Gastrocnemius medialis (%; upward phase)	129 ± 34 [105; 153]*	128 ± 42 [99; 157]	100 ± 0	**0.022**	*0.18*
Vastus lateralis (%; upward phase)	99 ± 25 [80; 118]	107 ± 21 [91; 123]	100 ± 0	0.64	

** Significantly different from athletic track. A negative value of length changes corresponds to shortening. Values are presented as mean ± standard deviation SD [confidence interval CI 95%]. Statistical significance was set at $P < 0.05$. Partial eta-squared η^2 is a measure of the effect size with $\eta^2 < 0.06$ considered as a small effect size, $0.06 < \eta^2 < 0.14$ a medium effect, and $\eta^2 > 0.14$ a large effect. Muscle excitation is expressed as a percentage of the average root-mean-square values obtained for both phases (i.e., downward and upward) on the athletic track. Thus, muscle excitation amplitude on athletic track corresponds to 100%.*

During the subsequent push-off phase, GM and VL fascicles actively shortened until take-off without being affected by surface properties (P = 0.90 and P = 0.94, respectively; **Figures 2C,D** and **Table 2**). However, we observed a significant higher GM activation during push-off on the artificial turf (P = 0.043) compared to the athletic track while there was a trend for a greater activity also on the hybrid turf (P = 0.052) (P = *0.022*; η^2 = 0.18, *large*, **Table 2** and **Figure 2A**). On the contrary, no difference was reported in VL muscle excitation between surfaces during the downward phase (P = 0.31) and push-off phase (P = 0.64). For both muscles, pennation angle peaked prior to take-off and did not differ between surfaces (**Figures 2G,H** and **Table 2**; P = 0.17 and P = 0.015, for GM and VL, respectively). Similarly, the type of surface did not influence the changes in pennation angle (**Table 2**; P = 0.09 and P = 0.014, for GM and VL, respectively). Average GM pennation angle between 80 to 85% of the CMJ was not statistically different between surfaces (P = 0.09; **Table 2** and **Figure 2G**) despite a trend for greater pennation angle on artificial turf compared to hybrid turf and the athletic track at this specific moment of the task (∼11–15%; η^2 = 0.19, large). Neither GM and VL peak (P = 0.13 and P = 0.65, respectively, **Table 2**) and average (P = 0.16 and P = 0.39, respectively, **Table 2**) fascicle shortening velocity were influenced by the type of surface. GM MTU started to shorten between 75 and 80% of the CMJ (**Figure 2E**) and shortened with the same amplitude between surfaces (P = 0.68), but at a different shortening velocity (P = 0.042; η^2 = 0.10, *medium*). Specifically, we observed a greater peak shortening velocity of the GM MTU on artificial turf (P = 0.042) and a trend for a greater velocity on the hybrid turf (P = 0.053) compared to the athletic track (**Table 2**). During push-off, VL MTU shortening and peak shortening velocity were similar between surfaces (P = 0.62 and P = 0.27, respectively, **Figure 2F** and **Table 2**). MTU gearing of the GM revealed a ∼4-fold greater MTU velocity than fascicles but was unaffected by surface properties (P = 0.20; **Table 2**). This MTU gearing ratio was much lower for the VL (∼1.8-fold) and remained similar between surfaces (P = 0.78; **Table 2**).

DISCUSSION

Despite different measurable surface mechanical properties, we observed no difference in maximal vertical jumping performance between surface conditions. We also found no influence of surface properties on muscle fascicle behavior of both GM and VL muscles during maximal countermovement jumping. However, we observed that surface properties altered GM muscle activation amplitude, which was higher on more absorbing and deformable surfaces such as turf. In addition, the shortening velocity of GM MTU achieved during the push-off phase of the jump was higher on artificial turf and hybrid turf than on athletic track. These adjustments ultimately resulted in greater peak ankle plantar flexion velocity on those surfaces compared to the stiff and less deformable athletic track, and may partially explain neuromechanics' regulation during jumping to offset changes in surface viscoelastic properties (e.g., increase in damping and compliance).

Surface Effects on Jump Performance and Kinematics

In accordance with recent studies on both indoor and outdoor sport surfaces (Malisoux et al., 2017; Firminger et al., 2019; Hatfield et al., 2019), jump height was not affected by the type of surface during CMJs. A recent study reported no difference between two different natural turf, an artificial turf, and a force plate (Hatfield et al., 2019) during vertical jumping. These results suggest that the differences in surface mechanical properties between common sport surfaces has only a marginal effect on CMJ performance. This is probably because of a low amount of surface deformation and energy exchange between the human body and the surface, resulting in minor additional work done by the surface, as previously observed in sprinting (Stafilidis and Arampatzis, 2007). We found a greater deformation capacity of the artificial turf compared to the hybrid turf and the athletic track. This can come from different combination of viscoelastic properties and express a global mechanical behavior rather than truly emphasize how surface damping and/or compliance increased. Nonetheless, turf surfaces are certainty more compliant and shock-absorbing than the athletic track. In addition, the higher energy restitution and vertical deformation values of the artificial turf compared to the hybrid turf suggests that the former surface is more deformable and elastic while the latter is more viscous (71% energy loss for hybrid turf vs. 62% energy loss for artificial turf). However, these differences did not impact vertical jump performance.

During CMJ, we observed a proximal-to-distal joint sequence with similar order of lower limb joints extension between surfaces. Our results suggest that jumping coordination remains similar between surfaces. This robust pattern of coordination between the main lower limb joints during jumping is also present when CMJ are performed on stiffer, steel-made force plate or soft and highly deformable sand surface (Giatsis et al., 2018). Similar joint range of motion (i.e., flexion and extension) and time to perform the preparatory countermovement and push-off were found between surfaces, which indicate that participants kept the same jumping strategy whatsoever the type of surface. However, we observed an increase in peak ankle plantar flexion velocity (i.e., reached ∼35 ms before toe-off) on the hybrid turf (+7 ± 9%; significant) and artificial turf (+6 ± 8%; non-significant) compared to the athletic track (**Table 1**). This higher ankle angular velocity could be possible because of smaller resistance at ankle plantar flexion on the more deformable surfaces (Giatsis et al., 2018). Previously, Giatsis et al. (2018) showed that a decreased resistance due to an increase in surface compliance resulted in both a larger ankle range of motion and angular velocity during CMJ on sand compared to a stiff surface. In our study, it is likely that the greater deformation capacity of the turf surfaces and probably the decreased resistance compared to the athletic track account for the higher peak ankle angular velocity late in the jump. However, the similar ankle range of motion observed between the three surfaces tested suggests that

ankle joint excursion is more affected when jumping on a range of very soft surfaces.

Surface Effect on Muscle Activity

We observed an increase in GM EMG amplitude in both phases when jumps were realized on the more deformable surface with large effect sizes (i.e., artificial turf) (**Table 2** and **Figure 2A**). The mean EMG activity of the GM muscle was on average ∼55% higher on the artificial turf compared to the athletic track, indicating a greater muscle excitation during the countermovement phase. During the first half of push-off, the mean EMG activity of the GM was also approximately 29% and 28% higher on artificial turf and hybrid turf, respectively, compared to athletic track. This result suggests that GM muscle may be more activated in response to an increase in surface deformation capacity (e.g., damping and/or compliance) during maximal vertical jump. It is possible that GM activity is high but submaximal during maximal CMJ, and that there is a potential for further EMG increase through recruitment of additional motor units in order to offset surface properties (Bobbert and van Ingen Schenau, 1988; Kurokawa et al., 2003). Thus, an increase in GM activity may further stiffen the muscle and could be seen as a neural strategy to adjust leg mechanics, and specifically increase ankle stiffness, on a range of compliant surfaces (Moritz et al., 2004). However, further research is needed to explain by which exact mechanisms GM EMG activity increased. VL muscle excitation was similar between surfaces during the countermovement and push-off phases (**Figure 2B**). Considering similar VL fascicle lengthening during the eccentric part of the CMJ, VL inhibition levels may be comparable when jumping on the three surfaces and could be a reason for the similar level of muscle excitation reported in this phase (Aagaard et al., 2000). In addition, while speculative, VL muscle activity may be near its maximal activation level during the push-off phase of the CMJ, as previously suggested by Nikolaidou et al. (2017). These authors showed that VL fascicles first operated toward optimal length for force generation at the beginning of the push-off phase. Then, when the muscle shortens, it develops high force and mechanical work at a high level of activation (Nikolaidou et al., 2017). In our study, VL fascicle length at the beginning of the push-off phase was similar between surfaces and would not have affected the level of VL activation. Therefore, although we did not assess maximal muscle activity in isometric conditions, further increase in VL muscle excitation may not be possible on more compliant surfaces like turf. Interestingly, we observed no difference in muscle activation/deactivation timings with no longer movement time. This means that the time the muscle actively produces force and work was similar between surfaces. If neural and/or joint coordination or timing were affected by surface properties, it could have consequences for energy flow between muscle and tendinous tissue and potentially affect jumping movement (Sawicki et al., 2015; Reynaga et al., 2019). Our hypothesis is that this is not the case in humans when jumping over these three sport surfaces. In comparison, (Wade et al., 2019) recently demonstrated that adding mass during CMJ increased the time to perform the movement, resulting in lower shortening velocity of gastrocnemius lateralis and soleus muscles, and probably increasing their force and work generation without increasing mean or maximal EMG amplitude. In our study, we could speculate that surface properties tend to affect GM muscle activation level rather than altering the timing of muscle activation and/or the time to perform CMJ. This is also in line with our previous study on the same three surfaces, where we observed an increase in EMG amplitude but no difference in timing of muscle excitation (Hollville et al., 2019). A recent study in animals showed a longer timing of muscle excitation on more compliant habitats, which caused a disruption of the energy flow between the environment and the body, and in turn jumping performance (Reynaga et al., 2019). Any potential changes in muscle excitation timing could differently tune muscle-tendon mechanics and jump height (Sawicki et al., 2015). This may also be a reason to explain the similar muscle fascicle behavior observed in our study. One could wonder how these timings of muscle excitation are affected in humans when jumping from very damped or elastic surfaces, and how it could affect muscle-tendon mechanics and jumping coordination through the proximal-to-distal joint sequence (Ferris and Farley, 1997; Arampatzis et al., 2004; Moritz et al., 2004).

Surface Effect on Muscle Fascicle Mechanics

Gastrocnemius medialis fascicles were decoupled from the MTU behavior (fascicles were quasi-isometric then shortened whereas MTU lengthened then was quasi-isometric then shortened) (Kurokawa et al., 2003) while we observed that VL fascicles' behavior (lengthening then shortening) was in phase with the MTU during jumping (Nikolaidou et al., 2017; **Figures 2C–F**). This result highlights a proximo-distal gradient within the limb to power the jump (e.g., differences in muscle function, tendon compliance) (Roberts, 2016). The higher level of GM activation observed during muscle shortening on the artificial turf (**Figures 2A,C**) likely increased muscle stiffness increasing the stretch of the tendinous tissues, and in turn the amount of energy stored on this surface (Bosco et al., 1981; Anderson and Pandy, 1993; Farris et al., 2016). Subsequently, elastic energy was released by shortening of the tendinous tissues when GM muscle activity started to decrease (**Figure 2A**). At this moment, MTU started to shorten (**Figure 2E**) and the ankle was extended. This catapult-like mechanism potentially amplified mechanical power in GM muscle during push-off (Anderson and Pandy, 1993; Kurokawa et al., 2003; Farris et al., 2016) and may partially explain the increase in peak shortening velocity of GM MTU on artificial and hybrid turf compared to athletic track (**Table 2**). The higher peak ankle plantar flexion velocity on the hybrid and artificial turf compared to the athletic track observed in the present study might account for such increase in ankle power output. However, it is not clear how there could be an increase in tendinous tissues energy storage and release on turf surfaces. An appealing explanation is related to muscle shape changes and the fact that when a pennate muscle shortens, it radially bulges due to its isovolumetric properties. Such muscle bulging probably exerts forces that load connective tissues not only longitudinally, but also transversely (Azizi and Roberts, 2009; Eng et al., 2018). This biaxial loading likely modulates aponeuroses stiffness in

longitudinal and transversal plane (Azizi and Roberts, 2009; Eng et al., 2018). While speculative, additional energy could have been stored through transverse strain on the more deformable surfaces. In addition, the high velocity amplification or MTU gearing of the GM measured as the ratio between MTU and peak fascicle velocity (i.e., GM MTU velocity is ∼four times higher than GM fascicle velocity; **Table 2**) highlights the key role of tendinous tissues and fascicle rotation to maximize mechanical power during jumping (Alexander and Bennet-Clark, 1977; Anderson and Pandy, 1993). Indeed, this reduction in fascicle velocity is mainly governed by the elastic recoil of the compliant tendinous tissues and to the rotation of GM fascicle. This elastic energy release increases in the late phase of the jump when the force decreases, and thereby likely increases muscle belly thickness (**Figure 2G**; Kurokawa et al., 2003). While initial GM pennation angle was the same between surfaces at the beginning of the push-off phase, fascicle rotation during the push-off phase appeared to be on average ∼11% to 15% greater on artificial turf than on the other surfaces at the time where tendinous tissues began to release elastic energy (**Figure 2G** and **Table 2**). This relative larger (on average 2.2° to 3°) fascicle rotation on artificial turf compared to athletic track and hybrid turf, with similar fascicle length between surfaces, likely increased muscle thickness. The changes in fascicle rotation and muscle belly thickness are considered to be determinants of belly gearing (Wakeling et al., 2011). While speculative, the trend for a greater fascicle rotation and likely muscle thickness observed on artificial turf could result in higher belly gearing on this surface. Such mechanisms may contribute to greater MTU velocity, and to a lesser extent MTU gearing. No significant difference in fascicle length changes and velocity suggest similar muscle fascicle operating length and shortening velocity between surfaces. Previously, Kurokawa et al. (2003) estimated that GM sarcomeres operated on favorable portion of the force-length curve (i.e., over the plateau and upper part of the ascending part) during CMJ, "where fascicles could generate more than 75% of the maximal" fascicle force (Kurokawa et al., 2003). Previous findings in vertical jumping suggest that the difference in muscle architecture and function between the monoarticular soleus and the biarticular GM and gastrocnemius lateralis, both contributing to the generation of ankle power during the push-off phase of the CMJ, may result in different fascicle behavior and elastic mechanisms (Kurokawa et al., 2003; Farris et al., 2016; Wade et al., 2019). It is also possible that soleus fascicle behavior was affected on the more deformable surfaces and contributed to the greater ankle plantar flexion velocity observed in this study.

Vastus lateralis fascicle actively lengthened during the downward motion to resist to inertial and gravitational forces (Nikolaidou et al., 2017). The countermovement allows the VL to produce more positive work during the subsequent push-off phase thanks to the increasing level and development of muscle force (Anderson and Pandy, 1993; Bobbert and Casius, 2005; **Figure 2B**). This mechanism is attributed to the pre-stretch potentiation of the VL and the ability of a muscle to produce more force after being actively stretched (Bosco et al., 1981), as well as the active muscle state during the preparatory countermovement (Bobbert and Casius, 2005). VL fascicles' behavior during jumping is similar to a previous study (Nikolaidou et al., 2017; **Figure 2D**), with high active lengthening and shortening in fascicle and MTU but without difference in magnitude between surfaces (**Figures 2D,F** and **Table 2**). In addition, pennation angle decreased throughout the countermovement phase and increased during the push-off until take-off (**Figure 2H**). However, we found no influence of surface properties (**Table 2**). This is reliable to the maximal muscle activity assessed on each surface (see previous paragraph) and consistent with previous observations showing that VL fascicles are crucial contributors to the positive mechanical work generation during push-off (Hubley and Wells, 1983; Nikolaidou et al., 2017). Moreover, a previous study during CMJ (Nikolaidou et al., 2017) demonstrated that mean VL fascicle shortening velocity is likely to be close to the plateau of the power–velocity curve and consequently has favorable average power potential. Our current findings show that mean VL fascicle shortening velocity is similar between surfaces and indicate that the sport surfaces tested did not induce changes in VL fascicle mechanics.

In our study, albeit speculative, for similar GM and VL force-length-velocity potentials and contraction history between surface conditions, the overall greater GM EMG activity observed on turf surfaces during the push-off phase suggest a greater GM contractile force output when jumping on artificial turf and hybrid turf compared to athletic track. This would partly explain the similar performances when jumping from more deformable surfaces with higher energy loss potential, likely primarily affecting distal joints and decreasing jumping efficiency (Stefanyshyn and Nigg, 2000; Reynaga et al., 2019).

Limitations and Methodological Considerations

The results of our study must be considered in light of the following limitations. First, we did not perform *a priori* sample size calculation, which prevents us from determining the power of our sample size. We thus provided confidence intervals (±95%) and effect sizes to better interpret the power of our analyses. Overall, we observed medium to large effect sizes for EMG activity, MTU shortening velocity, and ankle plantar flexion velocity. In addition, our results are clearly supported by previous findings in the field. However, our interpretations/conclusions would be strengthened with a larger sample size. This is especially true regarding non-significant *post hoc* found for ankle peak plantar flexion velocity on artificial turf compared to the athletic track, GM EMG activity during push-off, and GM MTU peak shortening velocity on hybrid turf compared to the athletic track. But maybe more interestingly, this may also hold for non-significant difference observed in GM peak/mean fascicle shortening velocity between surfaces with a small effect size (−11 to −15% less GM fascicle shortening velocity on artificial turf compared to athletic track and hybrid turf; P = 0.13–0.16; η^2 = 0.03–0.04), and GM pennation angle with a large effect size (∼11 to 15% greater pennation angle on artificial at 80 to 85% of the CMJ; P = 0.09; η^2 = 0.19). One could hypothesize that with a bigger sample size, GM fascicle shortening velocity may be slower and GM pennation angle greater during the push-off phase on artificial turf compared to the two other surfaces. Thus, a slower fascicle shortening velocity along with the similar fascicle length and higher EMG activity found would probably enable increased

force production on this surface to compensate for greater surface deformation. This could also suggest that an increase in surface deformation may further affect the interplay between GM fascicle and tendinous tissue. These hypotheses need to be confirmed in further studies. Second, we did not use three-dimensional (3D) motion capture system outdoors because of the non-optimal conditions of light for the use of infrared cameras, as well as the need to move our setup two times (from surface to surface) during an entire experiment. However, previous studies reported good agreement between 2D and 3D methods for lower body kinematics and kinetics during jumping (Hickox et al., 2016). Since the CMJ task is mainly restrained to the sagittal plane, we assume that 2D high speed video is appropriate to appraise the influence of different surfaces on 2D joint kinematics (Hickox et al., 2016). Third, we used a simple foot model to capture ankle kinematics, which is then used to compute MTU length. This simple model considers the foot as single rigid-body segment and could have influenced ankle joint and velocity data (Zelik and Honert, 2018). In addition, these estimations failed to account for variability between subjects, and recent studies highlighted their limits of use to estimate tendon work (Zelik and Franz, 2017; Matijevich et al., 2018). Indeed, given the three-dimensional nature of the muscle contraction, the use of 2D ultrasound and anthropometric models to appraise a 3D phenomenon is not without limitations (Cronin and Lichtwark, 2013; Roberts et al., 2019). For example, when a muscle is bulging under contraction, the present simple 2D models may not fully capture transverse strain of the aponeurosis, which partly accounts for longitudinal tendinous tissue length misestimations (Matijevich et al., 2018; Roberts et al., 2019). In this study, we did not estimate tendinous tissue lengths and rather focused on direct estimations of fascicle length to interpret GM and VL tendon function. Additionally, these methods only hold true when the muscle fascicle acts in the same 2D plane as the ultrasound image, thus possibly resulting in underestimation of muscle fascicle length changes when there is out-of-plane muscle motion (which we cannot fully rule out). Recent studies using freehand 3D ultrasound allowed to better understand such dynamic shape changes in skeletal muscle and tendinous tissues (Farris et al., 2013; Raiteri et al., 2018). However, it remains impossible to apply these methods in fast dynamic motor tasks such as jumping. Fourth, we used a fascicle-tracking algorithm previously validated for GM and soleus muscles (Cronin et al., 2011; Gillett et al., 2013; Farris and Lichtwark, 2016), but not VL, for which the validation remains to be done. Due to the small field of view used by the probes to estimate fascicle kinematics, extrapolation of the visible part of the fascicle was done according to aponeuroses motion (Kurokawa et al., 2003). Especially for VL muscle, fascicle length was systematically extrapolated (Brennan et al., 2017). In a pilot analysis ($N = 1$), we found that when using a single short probe, dividing VL fascicle length by a reference VL fascicle length estimated on a quiet standing trial allowed to reduce the percentage of fascicle length extrapolation to 3 to 4% in comparison to VL fascicle length measured with a dual probe arrangement (∼20%; Hollville et al., 2019). This is due to the fact that VL fascicle length value recorded during the static trial was systematically higher using one probe than using two probes which thus reduced the influence of overestimation when using one probe. We found no difference of GM and VL fascicle length changes when using both absolute and relative length values (**Table 2**). Eventually, the recent study of Brennan et al. (2017) also suggests that the use of a single probe method to estimate VL fascicle length and compare conditions performed in one experimental session is appropriate because of similar differences in muscle contraction dynamics within-participants. Fifth, we restricted the use of arm movement during CMJ in order to mainly examine the contribution of lower-limbs to power the jump. While all participants were familiar with performing maximal vertical jumps, this may appear as a novel task for some of them. However, we ensured that CMJ performance reached a plateau (i.e., no further increase in jumping height from trial to trial) during the familiarization by providing feedback about the jumping technique (jumping with arms restricted, trunk and legs fully extended during flight time until ground contact) and flight distance (i.e., maximal height reached in the air). This was confirmed by the good repeatability of performance whatever the surface during the actual testing (CV $< 5\%$). Sixth, we used the flight-time method to estimate flight distance. This method does not account for the center-of-mass displacement before take-off, and thus underestimates jump height. However, this method remains appropriate to estimate flight distance (Wade et al., 2020), especially outside laboratory conditions, and we observed low variability within-participants. Recent field-based estimates of jump height appear to be an interesting alternative of the use of the flight time method by adding the calculation of an anatomically scaled heel-lift constant to improve jump height estimation (Wade et al., 2020). Lastly, considering the unequal samples for fascicle related data ($N = 12$) and EMG data ($N = 10$ and 8 for GM and VL, respectively), caution should be made when interpreting these variables together.

CONCLUSION

We have provided evidence of slight adjustments in the mechanics of the gastrocnemius medialis muscle-tendon unit and muscle activation during maximal vertical jump on surfaces with different mechanical properties without modifying vastus lateralis behavior, jumping performance, and jump coordination. This suggests that the neuromechanics of the jump, especially at the distal joints level, can be affected by surface properties (i.e., increase in surface deformation capacity) during vertical jumping without altering jump height or coordination. These small alterations are mainly explained by a greater gastrocnemius medialis activity and a greater tendinous tissues and gearing contribution to the muscle-tendon unit shortening velocity and to the minor contribution of surface deformation, along with the similar jump coordination strategy used by the participants (e.g., proximal-to-distal joint sequence/neural coordination). Further investigations could extend this first study to a broader range of surface properties (e.g., Moritz et al., 2004) and movements (e.g., running, hopping, drop-jumping, Ferris and Farley, 1997; Kerdok et al., 2002; Arampatzis et al., 2004) in order to establish more general mechanisms about the relationship between surface mechanical behavior and muscle-tendon mechanics and neural control of movement.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by agreement no. 16/18 (Ouest IV). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors contributed to conception and design of the study, edited, revised, and approved the final version of the manuscript. EH performed the experiment, processed the data, and wrote the first draft of the manuscript.

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Impact of Altered Gastrocnemius Morphometrics and Fascicle Behavior on Walking Patterns in Children With Spastic Cerebral Palsy

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Spastic cerebral palsy (SCP) affects neural control, deteriorates muscle morphometrics, and may progressively impair functional walking ability. Upon passive testing, gastrocnemius medialis (GM) muscle bellies or fascicles are typically shorter, thinner, and less extensible. Relationships between muscle and gait parameters might help to understand gait pathology and pathogenesis of spastic muscles. The current aim was to link resting and dynamic GM morphometrics and contractile fascicle behavior (both excursion and velocity) during walking to determinants of gait. We explored the associations between gait variables and ultrasonography of the GM muscle belly captured during rest and during gait in children with SCP [n = 15, gross motor function classification system (GMFCS) levels I and II, age: 7–16 years] and age-matched healthy peers (n = 17). The SCP children's plantar flexors were 27% weaker. They walked 12% slower with more knee flexion produced 42% less peak ankle push-off power (all $p < 0.05$) and 7/15 landed on their forefoot. During the stance phase, fascicles in SCP on average operated on 9% shorter length (normalized to rest length) and displayed less and slower fascicle shortening (37 and 30.6%, respectively) during push-off (all $p \leq 0.024$). Correlation analyses in SCP patients revealed that (1) longer-resting fascicles and thicker muscle bellies are positively correlated with walking speed and negatively to knee flexion (r = 0.60–0.69, $p < 0.0127$) but not to better ankle kinematics; (2) reduced muscle strength was associated with the extent of eccentric fascicle excursion (r = –0.57, p = 0.015); and (3) a shorter operating length of the fascicles was correlated with push-off power (r = –0.58, p = 0.013). Only in controls, a correlation (r = 0.61, p = 0.0054) between slower fascicle shortening velocity and push-off power was found. Our results indicate that a thicker gastrocnemius muscle belly and longer gastrocnemius muscle fascicles may be reasonable morphometric properties that should be targeted in interventions for individuals with SCP, since GM

muscle atrophy may be related to decreases in walking speed and undesired knee flexion during gait. Furthermore, children with SCP and weaker gastrocnemius muscle may be more susceptible to chronic eccentric muscle overloading. The relationship between shorter operating length of the fascicles and push-off power may further support the idea of a compensation mechanism for the longer sarcomeres found in children with SCP. Nevertheless, more studies are needed to support our explorative findings.

Keywords: cerebral palsy, ultrasonography, triceps surae, muscle architecture, paresis, toe-walking, crouch gait

INTRODUCTION

Spastic cerebral palsy (SCP) is a neuromuscular disorder due to a nonprogressive brain lesion occurring early in infancy or before (Graham et al., 2016). As a result, patients with SCP often present hypertonia, hyperreflexia, and impaired motor control (Lee et al., 2014). Alterations of the musculoskeletal system, e.g., muscle weakness, restricted joint range of motion (RoM), and increased passive joint stiffness, are partly attributable to altered muscle-tendon properties (Barber et al., 2012). This likely contributes to limited mobility and restricted participation in daily life (Damiano et al., 2000).

Main intervention goals in the therapy of individuals with SCP are to reduce spasticity and to counteract the development and deterioration of muscular pathology. Thus, the plantar flexor muscle-tendon complex is frequently targeted during treatment, e.g., by physical therapy, surgeries, serial casting, orthoses, or botulinum toxin injections. In comparison to typically developing (TD) peers, plantar flexor muscles are macroscopically smaller and shorter in patients with SCP (Malaiya et al., 2007; Barber et al., 2011; Noble et al., 2014). For the gastrocnemius medialis (GM), reductions in muscle volume (Barber et al., 2013; Herskind et al., 2016), fascicle length (Barber et al., 2013; Hösl et al., 2015; Frisk et al., 2019), and physiological cross-sectional area (Barber et al., 2013; Herskind et al., 2016) have been frequently documented. In addition, tendon properties are changed, e.g., increased length (Wren et al., 2010; Hösl et al., 2015; Kruse et al., 2018) and reduced cross-sectional area (Gao et al., 2011; Kruse et al., 2018), have been reported. Examinations on microscopic level demonstrated longer sarcomeres (Lieber and Friden, 2002; Ponten et al., 2007; Smith et al., 2011; Mathewson et al., 2014; Mathewson and Lieber, 2015) and reduced serial sarcomere number (Lieber and Friden, 2018). These alterations likely limit muscle force output and excursion, which may in turn deteriorate walking.

Patients with SCP often walk slow, with increased energy demands (Kerr et al., 2008) and constrained or excessive joint movements with different gait characteristics (Armand et al., 2016; Zhou et al., 2017). Although the natural, untreated progression of gait pathology generally remains difficult to track in children with SCP, with higher age, crouch gait, a gait pattern with excessively flexed knees, might often increase (Bell et al., 2002; Gough et al., 2004; Rose et al., 2010). Crouch gait affects 45–60% of independently ambulant patients (Rethlefsen et al., 2017) with higher rates (74–88%) found in bilaterally affected patients (Wren et al., 2005), while equinus gait, characterized by ground contact with the forefoot or midfoot and subsequent lack of dorsiflexion, is present in nearly every second ambulant child with SCP. Concerning the force output during gait, the plantar flexors usually supply the major forward drive in healthy subjects, but during gait of patients with SCP, marked reductions in ankle joint power during push-off is a typical issue (Dallmeijer et al., 2011; Eek et al., 2011).

To date, there is yet a lack of information about the specific muscle properties in SCP that could be decisive for particular gait deviations. During gait, the plantar flexors generally control tibia progression, generate propulsion, and accelerate the forward swinging leg (Neptune et al., 2001; Arnold et al., 2005; Santuz et al., 2017). Distinguishing the gastrocnemius and soleus, both seem to similarly contribute to vertical support during unimpaired gait, but during the late stance phase the gastrocnemius induces forward acceleration while the soleus contributes to braking of forward velocity throughout mid- and terminal stance (Francis et al., 2013). Most notably, the gastrocnemius is a bi-articular muscle with the potential capacity to generate knee flexion upon stimulation, too (Lenhart et al., 2014). Overall, in SCP, a thinner GM muscle or triceps surae seems to be related to reduced gross motor function (Ohata et al., 2006; Choe et al., 2018). Moreover, the gastrocnemius muscle volume is smaller in children without independent walking skills (Herskind et al., 2016). In addition, plantar flexors of children with SCP show decreased concentric (Ross and Engsberg, 2002) or isometric strength (Wiley and Damiano, 1998; Elder et al., 2003; Stackhouse et al., 2005; Downing et al., 2009) during instrumented strength tests. Still, more detailed knowledge about the actual relationship of muscle structure to biomechanics of gait is needed. Fairly recently, Martin Lorenzo et al. (2018) captured passive gastrocnemius fascicle length with ultrasound aiming to explain the amount of reduced propulsive ankle joint power during gait (Martin Lorenzo et al., 2018). However, they could not find statistically significant associations. Frisk et al. (2019) speculated that reduced fascicle lengths are essential contributors to reduced torque generation in the gastrocnemius in SCP. They assumed that a more plantar-flexed position during gait (~more equinus) would take advantage of the underlying alteration of the torque-angle relation (Barber et al., 2012). Thus, children with shorter fascicles may opt to walk in larger plantar flexion to avoid unfavorable length-force relationships of their sarcomeres. However, the understanding may be compromised by a lack of knowledge

about the muscle fascicles' behavior during gait, which they did not simultaneously examine.

Ultrasound studies distinguished the muscle and tendinous behavior *in vivo*. They demonstrated that, in healthy heel-toe gait, the GM or soleus fascicles (Fukunaga et al., 2001; Ishikawa et al., 2005; Aggeloussis et al., 2010; Rubenson et al., 2012; Barber et al., 2017) shorten during loading response, the gait cycle's first period of double-limb support after initial ground contact. Thereafter, they display a subsequent active stretch-shorten cycle with near isometric or minor eccentric length change during single stance and shortening during push-off. During this cycle, the tendon stretches to a larger extent and stores elastic strain energy that is released during recoil. Since the tendon undergoes most of the whole muscle-tendon unit (MTU) length change, the muscles are able to contract at favorable lengths (at or near the plateau region of their force-length curve) and at a slower speed for force production (Fukunaga et al., 2001). The tendon stiffness appears crucial for the efficiency of this interaction (Lichtwark and Wilson, 2008).

Knowledge about the contractile mode of the spastic plantar flexors during gait has been often theory-driven, based on expertise (Gough and Shortland, 2012), forward simulations (Neptune et al., 2007), or musculoskeletal modeling (van der Krogt et al., 2015; Rha et al., 2016). While structural shortening of the spastic plantar flexor MTU derived from musculoskeletal modeling has been primarily linked with equinus gait (Eames et al., 1997), among other factors (Delp et al., 1996; Hicks et al., 2008), a shorter and passively less extensible gastrocnemius MTU is reflected by increased crouch gait overtime (Maas et al., 2015).

Fairly recently, ultrasonography was also used to investigate the *in vivo* walking behavior of the GM muscle in children with SCP (Hösl et al., 2016; Kalsi et al., 2016; Barber et al., 2017). However, the studies reported seemingly inconsistent results. Kalsi et al. (2016) found increased lengthening of the gastrocnemius muscle belly during single support in comparison to their TD peers. Barber et al. (2017) delivered evidence of increased fascicle lengthening during the early and mid-stance phase; however, contrastingly, Hösl et al. (2016) did not find increased lengthening excursions but less fascicle shortening during push-off in accordance with Barber (2017). The inability to resist tensile forces was assumed to result in energy absorbed by the muscle rather than stored by the tendon, potentially damaging the muscle on the long term, which was in line with earlier speculations about harmed fiber growth (Gough and Shortland, 2012) or promoted fibrosis (Pitcher et al., 2015) due to eccentric overloading in SCP. In this context, three aspects warrant further investigation: a different strength level may potentially be related to the extent of eccentric fascicle lengthening. Additionally, in the studies of Kalsi et al. (2016) and Barber et al. (2017), children also primarily presented alterations in the ankle joint (equinus gait), while in Hösl et al. (2016) patients walked on average with increased knee flexion. Since a steeper landing angle of the foot (i.e., more toe walking) may generally exert larger tensile forces on the gastrocnemius muscle-tendon complex, the walking pattern might play a crucial role. Moreover, healthy populations show a speed-dependent modulation of the contractile modes (Ishikawa et al., 2005), e.g., greater lengthening of the GM muscle belly during running than during walking (Ishikawa et al., 2005).

Evidence on the contractile fascicle behavior during gait and its relation to ankle kinetics is scarce. Barber et al. (2017) hypothesized that since muscle fascicles in SCP shorten less during late stance, they induce less ankle joint power. Also, Frisk et al. (2019) found less fascicle shortening during supramaximal stimulations simultaneously with reduced torque generation in SCP patients, but they were questioning if this would affect muscle force output during gait. Next to that, the implications of shorter relative operating length (Hösl et al., 2016) were not investigated. In TD individuals, the gastrocnemius fascicles usually operate at shorter relative length at faster walking (Ishikawa et al., 2005). This shift toward the ascending limb of the force-length relationship decreases muscle force output but may support the storage of elastic energy (Ishikawa et al., 2005) to augment the power output at the ankle joint during faster walking (Schwartz et al., 2008). Based on findings of longer sarcomeres and reduced sarcomeres in series in SCP (Lieber and Friden, 2002; Ponten et al., 2007; Smith et al., 2011; Mathewson et al., 2014; Mathewson and Lieber, 2015), the sarcomeres in SCP may likely need to operate with less overlap. Maintaining shorter fascicle operating length could thus be vital to produce push-off. Furthermore, fascicle shortening velocities have not yet been examined in SCP muscles. However, Farris and Sawicki (2012) stated that GM fascicle shortening velocity during stance may be a key factor for the speed healthy humans choose to walk at. Biomechanically, higher fascicle velocities during stance phase could actually impair the fascicles' ability to produce force.

In this study, we wanted to link resting GM muscle morphometrics and the contractile fascicle behavior (both in length and velocity) during walking in children with SCP to kinematic and kinetic determinants of gait and muscle function. Due to the neuromuscular impairment in children with SCP, we were also keen to see whether the relationships were the same as in healthy controls. We performed a further analysis of the sample presented in Hösl et al. (2016). Data of children with SCP and their TD peers captured during both rest and during gait were analyzed. Ankle joint kinetics were already presented by Barber et al. (2017); however, the associations of the fascicle contractile modes and gait kinetics were not established. We thus complemented the ultrasound-based contractile behavior of the gastrocnemius fascicles during gait by Hösl et al. (2016) with ankle joint kinetics from inverse dynamics during overground gait of the same participants. Three aims were deduced: first, we aimed to study the relationship between alterations in resting-muscle fascicle length as well as muscle thickness (MT) and joint excursion as well as muscle function, e.g., during push-off. We assumed that children with SCP with more severe muscle pathology, namely, shorter resting GM muscle fascicle lengths and thinner gastrocnemius muscle bellies, will walk slower and less erected (with more knee flexion) and provide less push-off power. With reference to Frisk et al. (2019), we also expected that children with shorter fascicles will walk in more plantar flexion. Second, we aimed to further explore the divergent findings on eccentric fascicle

lengthening in SCP during gait. We expected larger fascicle lengthening in weaker children with SCP, in those with a steeper foot landing angle (i.e., an index for more severe forefoot landing), and in slower walkers. The third aim of our study was to investigate if the fascicle dynamics, i.e., operating length, shortening excursion, or velocity, are related to reductions in ankle push-off power. We expected that a longer operating length, less fascicle shortening excursion, and faster shortening velocities will be related to smaller push-off power in the SCP and TD children. Eventually, due to its exploratory nature, this study aims to generate further research goals to be studied.

MATERIALS AND METHODS

In the following, a brief description of the participant characteristics and study protocol can be found. For detailed information, we refer to Hösl et al. (2016).

Participants

Fifteen children with bilateral SCP (mean age: 11 ± 2.8 years, four females) and 17 TD controls (mean age: 12.2 ± 2.3 years, eight females) participated in the study. Eleven children with SCP were classified as GMFCS level I and four children as GMFCS II. Exclusion criteria were any leg surgeries or botulinum toxin injections within the last 12 months. Only data of the more involved side (i.e., less passive dorsiflexion) were included. Further anthropometric details can be found in Hösl et al. (2016). For the TD controls, the right side was chosen. Experiments received ethical approval by the Technical University Munich, Germany, and informed written consent was obtained (Hösl et al., 2016).

Protocol

The participants were physically examined (for passive RoM and modified Ashworth scale [Bohannon & Smith, 1987] at the knee and ankle) and performed isometric strength tests and a 3D gait analysis on a treadmill, as well as during overground walking. During the physical exam, passive dorsiflexion was measured using ruler-based goniometry. Moreover, a seated rest measurement was performed with the knees 90° flexed and ankles in neutral alignment (foot flat on the ground) to assess the GM muscle morphometrics at rest and extract the resting length of fascicles. Subjects were encouraged to relax and based on their feedback that they did not feel any tension. Although influenced by hypertonia and contracture, the modified Ashworth scale was used as a surrogate measure of spasticity. Since we did the resting measurement once with electromyography (EMG) and once with ultrasound (Hösl et al., 2016), we can also confirm that the muscle was silent. Peak isometric plantar flexor force (N/kg) was assessed by use of a handheld dynamometer and a uniaxial force sensor (Mobi, Sakaimed, Tokyo, Japan) during five maximum voluntary contractions (MVC, 3-s contraction, 1 min rest). For these, participants were long-seated (hips semi-flexed, knees extended, and ankle as close as possible to neutral). After discarding the lowest and highest values, three trials were averaged. No motion capture data were collected during the strength tests. For the current analysis, data captured during the flat-forward walking on both the treadmill and overground were included (Hösl et al., 2016). Since the treadmill was un-instrumented, the gait kinetics were extracted from the overground walking trials.

Gait Analysis

In both conditions, gait analyses were performed at the children's preferred walking speed. A Nexus system (Vicon Inc., Oxford, UK) with eight MX-Cameras was used to capture lower-limb kinematics using a modified plug-in gait model (Stief et al., 2013). Marker data were sampled at 200 Hz. On the treadmill (Atlantis, Heinz Kettler, Ense-Parsit, Germany), the participants wore a harness (h/p/cosmos, Nussdorf-Traunstein, Germany) without weight support, which was connected to a safety frame (Mobil Konzept Loadmaster 80, RehaMed Technology, Dietzenbach, Germany). After a familiarization period, data were captured during 10 s of treadmill walking. During the overground condition, the children walked up and down a 12-m-long flat walkway and ground reaction force data were captured at 1000 Hz *via* two force plates (AMTI, Watertown, United States). Three or more consistent kinetic trials were averaged.

Ultrasound and Electromyography

An Echoblaster 128 ultrasound (Telemed, Vilnius, Lithuania) was used to image the GM muscle fascicles at 60 Hz with a linear probe (8 MHz, field of view of 60 mm) during gait (**Figure 1**). The probe was held in place with a plastic cast covered with neoprene. Measurements of the fascicle lengths were made at a mid-belly position (i.e., half-way between muscle-tendon-junction and popliteal crease), and the scanner was aligned according to Benard et al. (2009). The ultrasound was synchronized with the motion capture data *via* a pulse that was fed to a DTS EMG System (Noraxon, Scottsdale, United States). Surface electrodes (Blue Sensor N, Ambu, Ballerup, Denmark) were placed on the muscle bellies of the GM, soleus, and tibialis anterior, and signals were sampled at 1000 Hz. EMG and ultrasound were captured in separate trials. A sequence of six successive strides was cropped into separate gait cycles. Ultrasound videos of each gait cycle were then analyzed separately. Fascicle lengths were measured with a tracking algorithm (Gillett et al., 2013), and subsequent frame-wise inspection by the same investigators (AZ and MH) and corrections were manually made were necessary. Gait data were interpolated to 100 points across each stride, and an average for each participant and condition was determined.

Furthermore, resting-muscle measurements were performed during seated rest defined with the knees in 90° flexion and the ankle in a neutral position (foot flat on the ground). In addition, the muscle-belly thickness was measured in the same resting position. During gait, all morphometric variables

FIGURE 1 | Example of a child with SCP during treadmill gait and the ultrasound probe fixated at the calf and markers from 3D motion capturing. The safety harness (h/p/cosmos, Germany) did not provide bodyweight support. The ultrasound probe Echoblaster 128 ultrasound (Telemed, Vilnius, Lithuania) was attached with a custom-made plastic cast, covered with neoprene, and firmly attached to the shank with Velcro straps, halfway between popliteal crease and the gastrocnemius muscle-tendon junction (MTJ).

were normalized to the resting lengths and all resting values were normalized to shank length. Further information about the post-processing e.g., calculation of MTU length (Orendurff et al., 2002) and EMG processing (Panizzolo et al., 2013) can be found elsewhere (Hösl et al., 2016). However, the information about GM MTU length and EMG activity was included for illustrative purposes in the present study.

Outcomes

Self-selected walking (SSW) speed was presented as absolute values (m/s). For kinematics, we extracted the foot landing angle (i.e., foot to floor landing angle at initial contact), the mean dorsiflexion, and knee extension during stance. Concerning joint kinetics during gait, joint moments were normalized to body mass (Nm/kg). We further extracted the positive peak ankle joint power (W/kg) during push-off. For the gastrocnemius morphometries, we selected the resting muscle belly thickness and fascicle length during seated rest. For quantifying the working mechanisms during gait, we calculated the mean fascicle operating length (% resting length) in stance, as well as the extent of eccentric excursion during single stance and the concentric excursion during push-off. Furthermore, the respective shortening velocity was calculated during push-off (expressed in % resting length/s). We further computed the mean isometric plantar flexor strength (N/kg) from three trials as described above.

Statistics

Participant characteristics, physical exam results, and muscle morphometrics during rest and gait were compared between children with SCP and TD children by use of unpaired *t*-tests or Mann-Whitney U tests if the variables were not normally distributed. With regard to the associations, we correlated the resting muscle belly thickness and fascicle lengths with the SSW, the kinematics, and the peak push-off power. For eccentric lengthening, we tested the correlations to isometric plantar flexor strength, the landing angle of foot, and the SSW. For dynamic fascicle behavior, we tested the associations of relative fascicle operating length, fascicle shortening excursion, and fascicle shortening speed to the peak power during push-off. All bivariate relationships were tested both for SCP and for TD children by use of Pearson correlations or, if not normally distributed, by Spearman-rank correlations. Correlation coefficients were interpreted as poor (<0.2), fair (0.21–0.4), moderate (0.41–0.6), and good (0.61–0.8) following the guidelines by Altman (1990). Normal distribution was tested with Shapiro-Wilk tests. The alpha level was set to 0.05 for group comparisons and during correlations corrected for familywise comparisons according to Šidák (1967) as follows: $\alpha_{sid} = 1 - (1 - 0.05)^{1/K}$, with K being the number of dependent variables for each set of correlations. Thus, the Šidák-adjusted level (α_{sid}) was 0.0127 for correlations with resting morphometrics and 0.0170 for all other sets of tests. If directed hypotheses were formulated, one-tailed tests were performed.

RESULTS

The findings are displayed as means ± standard deviations. Compared to their TD peers, children with SCP displayed significant reductions in passive dorsiflexion when measured with extended knees during the clinical exam (1 ± 8° and 15 ± 4° in SCP and TD, respectively, $p < 0.01$) accompanied by a modified Ashworth scale score of 2 ± 0.8 (range 1–3).

Their reference fascicle lengths and muscle-belly thickness, both measured during seated rest, were reduced by 18% (both $p \leq 0.016$). An example of an ultrasound image for both groups is shown in **Figure 2**.

During overground walking, the self-selected speed was 1.15 ± 0.17 m/s in SCP and 1.31 ± 0.14 m/s in TD ($p < 0.01$). With respect to the overground walking, the decrease in comfortable speed on the treadmill was similar ($p = 0.71$) between the TD children and the children with SCP (5 ± 10% and 6 ± 10%, respectively).

Children with SCP walked in a more crouched posture than their TD peers, characterized by significantly ($p = 0.02$) increased knee flexion (+6°) and slightly higher dorsiflexion (+1°) during stance (**Figures 3A,B**). The resulting kinematically

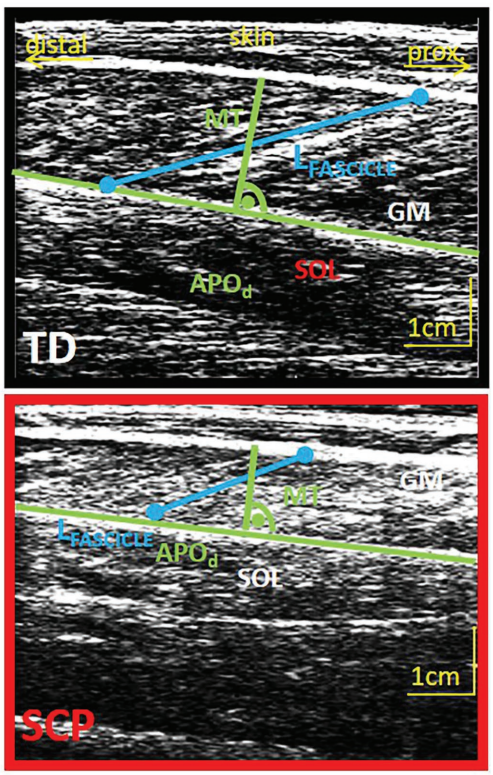

FIGURE 2 | Example of an ultrasound image of the GM muscle belly of a TD child (12 years, upper image) and a child with SCP (13 years, lower image) and schematic extraction of parameters of interest. Green: determination of muscle thickness (MT) at the midbelly portion perpendicular to the deep aponeurosis. Blue, fascicle length; APO_d, distal aponeurosis; GM, gastrocnemius medialis; SOL, soleus.

modelled MTU length is shown in **Figures 3E**. In addition, seven out of 15 children with SCP landed with their forefoot first. Concerning the ankle moment (**Figures 3C**) and the ankle joint power (**Figures 3D**), the peaks during push-off were reduced by 20.5% and 42.0%, respectively (both $p < 0.01$).

The children with SCP reached 9% ($p = 0.04$) shorter maximum fascicle lengths (**Figure 3F**) and showed 37% less concentric fascicle shortening during push-off (**Figure 4A**, $p = 0.001$) with no significant group differences in eccentric fascicle excursion during stance (**Figure 4A**, $p = 0.57$).

Furthermore, the peak fascicle shortening velocity (**Figure 3G**) during push-off was 30% slower in the children with SCP ($p < 0.01$).

Figure 3H displays the simultaneous electromyographic muscular activity. Moreover, the isometric strength of the children with SCP was decreased by 27% (**Figure 4B**, $p < 0.01$). Resting fascicle lengths showed a significant positive correlation of good strength with SSW speed in children with SCP (**Table 1**, r = 0.61, $p = 0.008$). Furthermore, thicker muscle bellies were also significantly positively correlated with walking speed (**Table 1**, $r = 0.60$, $p = 0.01$).

The moderate positive correlation between resting fascicle length and push-off power in children with SCP failed to reach statistical significance after Šidák correction (**Table 1**, $p = 0.042$). However, negative correlations of good statistical strength were found between longer fascicles as well as thicker muscle bellies and knee flexion (both $p \leq 0.004$) in children with SCP. The negative correlations between fascicle length as well as MT with the dorsiflexion angle failed to reach significance. No significant correlation between the resting-muscle morphometrics and the gait parameters was found in the TD children (**Table 1**, **Figure 5**).

Despite no significant group difference in eccentric fascicle excursion in children with SCP and TD (**Figure 4A**), there was a moderate negative correlation between the amount of eccentric excursion and the plantar flexor strength (rho = −0.57, $p = 0.015$) only in the patients with SCP (**Figure 4B**, **Table 2**), indicating that weaker children with SCP experienced larger fascicle lengthening during the stance phase of gait. The landing angle of the foot or walking speed did not show a significant correlation with the extent of eccentric fascicle excursion (**Table 2**).

Normalized operating length of the fascicles moderately negatively correlated with peak push-off power at the ankle joint in children with SCP (**Table 3**, **Figure 6A**, rho = −0.58, $p = 0.013$) indicating that the shorter the fascicles with respect to the resting length, the more push-off power may be generated. Furthermore, the fascicle shortening velocity or excursion did not show a significant correlation with the plantar flexion power during propulsion in the children with SCP (**Table 3**, **Figure 6B**), while in the TD children, a significant good association was found between slower shortening velocity of the fascicles and larger push-off power (**Figure 6B**, rho = 0.61, $p = 0.0054$). No significant correlation was found for SSW.

DISCUSSION

In this study, we aimed to link resting GM muscle morphometrics and the contractile fascicle behavior (both excursion and velocity) during walking in children with SCP to kinematic and kinetic determinants of gait and muscle function. One main finding was that longer resting-muscle fascicles and thicker muscle bellies were positively correlated with walking speed. Furthermore, our findings suggest that longer resting fascicles and thicker muscle bellies may contribute to less flexed knees but may not normalize ankle kinematics. In addition, our results may suggest that reduced muscle strength seems to be an important factor related to an increase in eccentric excursion by the spastic paretic muscle during gait. Finally, our analyses indicated that an association between shorter operating length of the fascicles and more push-off power at the ankle may exist.

Focusing on resting-muscle morphometrics, children with SCP with longer gastrocnemius fascicle lengths and thicker muscle bellies walked faster and, as hypothesized, more erected (i.e., with less crouch gait). Concerning fascicle lengths, these findings are somewhat in line with biomechanical

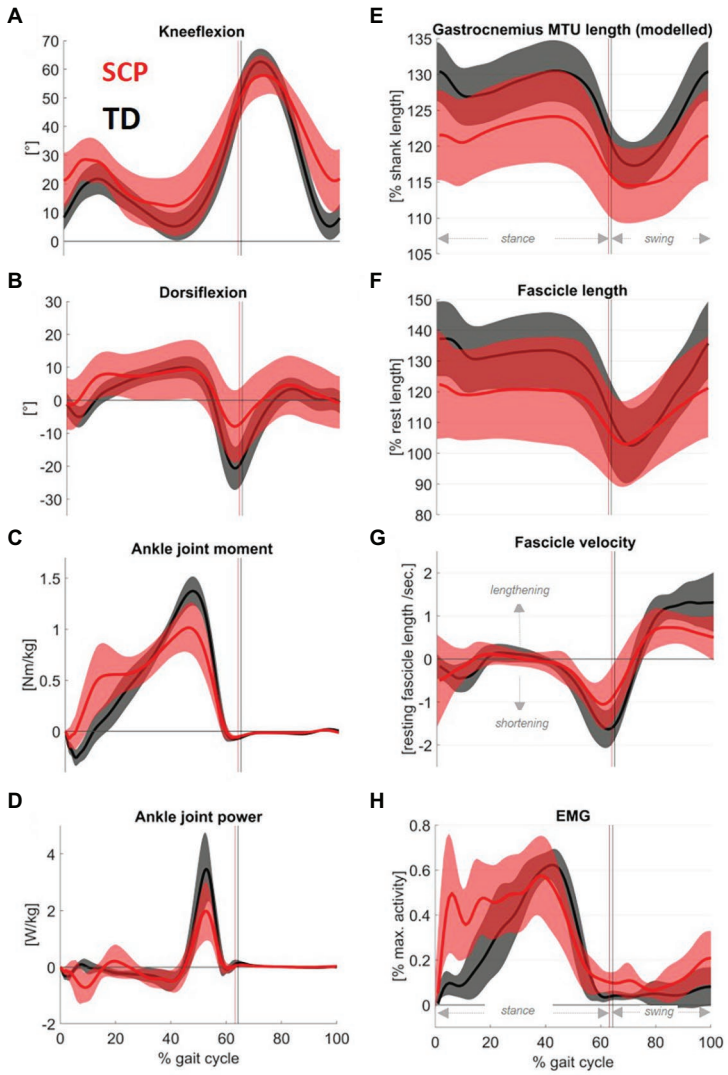

FIGURE 3 | Mean traces (shaded area: 1SD) of sagittal plane **(A)** knee and **(B)** ankle angles, as well as **(C)** ankle joint moments and **(D)** ankle joint power in children with SCP (red) and their TD peers (black). The figure further displays **(F)** the gastrocnemius fascicle length changes throughout the gait cycle and **(G)** the calculated fascicle velocity. For the sake of completeness, the graphs were complemented by **(E)** modeled GM muscle-tendon unit (MTU) length and **(H)** the gastrocnemius activity assessed with electromyography. Electromyography (EMG) was rectified, filtered, and normalized on the max. Activity of all walking trials for each individual.

advantages of longer fascicles, extending their range of active force production (Blazevich and Sharp, 2005; O'Brien, 2016). Since at a given fascicle shortening velocity, the shortening of each sarcomere is less in longer fascicles, relative contractile velocity is lower and its force potential can be larger (Blazevich and Sharp, 2005). In computer simulations, next to other muscles that mainly contribute to an erected gait, e.g., the soleus or the glutei (Hicks et al., 2008; Ong et al., 2019), also the gastrocnemius vertically accelerates the center of mass, thus contributing to a more upright gait (Steele et al., 2013). Moreover, additional sarcomeres in series may also increase the passive muscle extensibility (Butterfield, 2010). Longer gastrocnemius fascicles could thereby allow children with SCP to walk more erected. Whether longer fascicles contain more sarcomeres remains debatable in SCP; however, benefits of longer passive fascicle length have been reported previously in SCP: longer rectus femoris muscle fascicles were related to higher sports- and physical functioning scales (Moreau et al., 2010), whereby longer fascicle lengths in the tibialis anterior were significantly related to faster walking (Bland et al., 2011). Our finding therefore supports the importance of longer fascicles also for the function of the GM in individuals with SCP. However, in contrast to our expectation and therefore also in contradiction of the speculations by Frisk et al. (2019), we did not find a positive correlation between longer fascicle lengths and more dorsiflexion in children with SCP. Our hypothesis was therefore rejected.

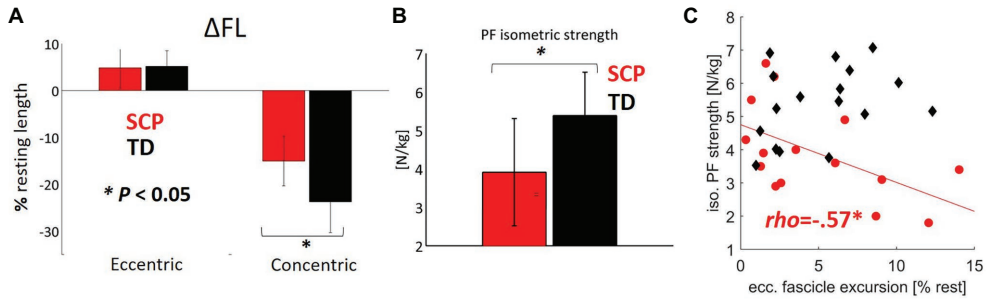

FIGURE 4 | (A) Extent of eccentric and concentric fascicle excursions (ΔFL calculated with regard to the resting fascicle lengths) in children with SCP (red) and TD children (black). **(B)** Plantar flexor strength. **(C)** Relationship between the plantar flexor (PF) strength and eccentric fascicle excursion. $^{*}p < 0.05$ for group comparisons concerning ΔFL and isometric strength. $^{*}p < 0.017$ for correlations (with Šidák-adjusted level). NB, only regression lines for sign. Correlations are shown for each group.

TABLE 1 | Correlation coefficients [Pearson's *r* (italic font) and Spearman's rho (upright font)] between gastrocnemius medialis (GM) muscle belly morphometrics at rest and gait-related outcomes in children with spastic cerebral palsy (SCP) and typically developing (TD) children.

Variables	Group	SSW (m/s)	DF (°)	KF (°)	PF power (W/kg)
Fascicle length (% shank length)	SCP	***0.61****	*−0.55*	***−0.66****	0.46
	TD	*0.02*	*−0.03*	*−0.38*	*0.03*
Muscle thickness (% shank length)	SCP	***0.60****	*−0.57*	***−0.69****	0.19
	TD	−0.12	0.11	−0.16	−0.16

$^{*}p < 0.0127$ *(with Šidák-adjusted level).*
SSW, self-selected walking speed; DF, mean dorsiflexion during stance; KF, mean knee flexion during stance; PF power, peak ankle joint power. Background color defines the strength of correlation <0.2, poor (red); 0.21–0.4, fair (yellow); 0.41–0.6, moderate (light green); 0.61–0.8, good (dark green). Significant values are also displayed in bold.

TABLE 2 | Correlation coefficients [Pearson's *r* (italic font) and Spearman's rho (upright font)] between GM eccentric fascicle excursion during gait and both plantar flexor (PF) strength and gait-related outcomes in children with SCP and TD children.

Variables	Group	PF force (N/kg)	SSW (m/s)	Foot landing angle (°)
Eccentric excursion (% resting length)	SCP	**−0.57***	−0.21	−0.08
	TD	*0.36*	*−0.35*	0.42

$^{*}p < 0.017$ *(with Šidák-adjusted level).*
PF force, maximum isometric strength from handheld dynamometry; SSW, self-selected walking speed. Background color defines the strength of correlation <0.2, poor (red); 0.21–0.4, fair (yellow); 0.41–0.6, moderate (light green); 0.61–0.8, good (dark green). Significant values are also displayed in bold.

TABLE 3 | Correlation coefficients [Spearman's rho (upright font)] between GM fascicle dynamics during gait and ankle joint push-off power (PF power).

Variables	Group	Mean operating length (% resting length)	Concentric fascicle excursion (% resting length)	Max. shortening speed (resting length /s)
PF power (W/kg)	SCP	**−0.58***	−0.01	-0.00
	TD	−0.23	−0.43	**0.61***

$^{*}p < 0.017$ *(with Šidák-adjusted level).*
Background color defines the strength of correlation <0.2, poor (red); 0.21–0.4, fair (yellow); 0.41–0.6, moderate (light green); 0.61–0.8, good (dark green).

More deteriorated muscular pathology of the gastrocnemius as indicated by a thinner muscle belly and shorter fascicles was associated with a more pronounced knee flexion. This may usually be noted as a sign of progressive functional decline in individuals with SCP (Bell et al., 2002). Musculoskeletal modeling showed that the MTU length of the gastrocnemius can be short in flexed knee gait (Rha et al., 2016) or in equinus gait (Wren et al., 2004). Both often lead to recession surgeries in SCP. Although treatments aim to restore dorsiflexion and not to normalize muscle-tendon architecture (Sees and Miller, 2013), it needs to be taken into account that gastrocnemius' fascicles or muscle belly thickness may undergo considerable atrophy due to an intervention as shown after Botulinum toxin application (Park et al., 2014), surgeries (Shortland et al., 2004), and splinting (Hösl et al., 2015). The associations found in this study may suggest that inducing gastrocnemius muscle atrophy due to treatment could help to correct excessive plantar flexion in children with bilateral SCP but may also cause a risk to induce undesired knee flexion during gait. With all due reservations considering the cross-sectional nature of this study, whether children show progressive crouch gait pathology could thus be influenced by preservation of muscle size.

Concerning the association between the gastrocnemius MT or the muscle fascicle lengths and peak ankle joint power, we could find a significant relationship in neither the individuals with SCP nor their TD peers. However, peak ankle power was reduced by −42% in the SCP group. Previous studies in healthy individuals showed that the active isometric plantar flexor force is proportional to their cross-sectional area (Fukunaga et al., 1996). In addition, the vastus lateralis MT was a good predictor of the voluntary knee extensor torque in patients with SCP and in TD children (Moreau et al., 2010). Still other studies reported no strong relationship between

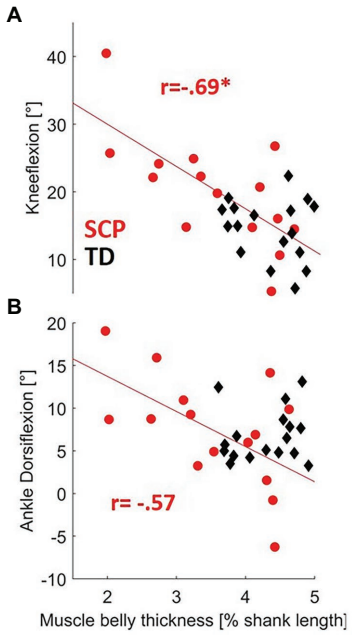

FIGURE 5 | Relationship between resting-MT and both **(A)** knee flexion and **(B)** dorsiflexion during gait in children with SCP (red circles) and TD children (black diamonds). $^{*}p < 0.0127$ (with Šidák-adjusted level). NB, regression lines for MT for SCP failed to reach significance to dorsiflexion ($p = 0.0284$).

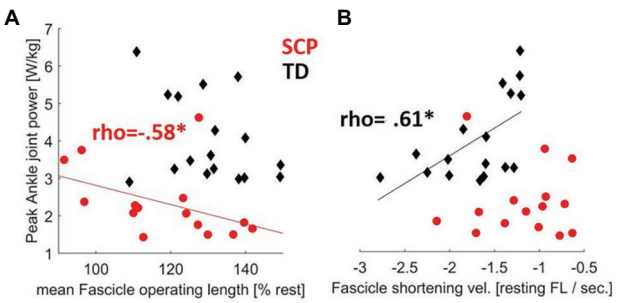

FIGURE 6 | Contractile determinants of push-off power: relationships between the **(A)** fascicle operating length during gait and the **(B)** fascicle shortening velocity during gait with the propulsive ankle joint power in children with SCP (red circles) and TD children (black diamonds). $^{*}p < 0.017$ for correlations (with Šidák-adjusted level). NB, only regression lines for sign. Correlations are shown for each group.

muscle size (i.e., muscle volume and anatomical cross-sectional area of the knee flexors and extensors) and strength in children with SCP (Reid et al., 2015). Since children with SCP display reduced specific tension (torque/unit CSA) compared to their TD peers, the association between plantar flexor muscle size and force output during gait may critically depend on neural recruitment issues (Elder et al., 2003). Yet, this has not been evaluated in this study. The absent relations in the TD children might reflect the rather homogenous and unconstrained gait patterns but could also reveal a greater dependency on their tendons for locomotion by using the passive elastic energy storage and recovery to reduce muscle work.

Regarding the divergent findings on eccentric fascicle lengthening, our results suggest that a low isometric strength level (e.g., also due to recruitment issues) may be related to the extent of eccentric excursion experienced by the gastrocnemius fascicles. Therefore, our finding is in line with the hypothesis as already formulated by Barber et al. (2017). In the past, the idea was raised that an eccentric overloading of the muscles may be a primary mechanism for an altered adaptation in connective tissue properties in children with SCP (Gough and Shortland, 2012), eventually negatively influencing factors such as the joint range of motion. In general, eccentric training appears to be beneficial for growth of healthy muscle fiber length *via* sarcomerogenesis (Lynn and Morgan, 1994; Butterfield et al., 2005) but perturbs fiber mechanics inducing myofibrillar remodeling (Carlsson et al., 2007; Franchi et al., 2014). Upon chronic eccentric overloading, insufficient repair process and substitution of myofibers by nonfunctional fibrotic tissue may be promoted (Zhu et al., 2007; Serrano and Muñoz-Cánoves, 2010). Our current finding supports the idea that weaker children with SCP may experience greater eccentric fascicle lengthening during gait. However, whether this actually overloads their muscles remains to be investigated. Nevertheless, we suggest focusing on efficient strengthening strategies for individuals with SCP.

We also analyzed the relationship of the foot landing angle (the steeper the more forefoot landing) and the extent of eccentric fascicle excursion. The current analysis demonstrated no significant correlation. Our sample might include less severe toes walkers than those included in the study of Barber et al. (2017); however, our finding agrees with previous studies that assessed the effect of voluntary toe-walking on the muscle-tendon behavior in healthy populations in which the stretch of the plantar flexors was fully taken up by the tendon (Lorentzen et al., 2018). Nevertheless, we did not examine the Achilles tendon (AT) behavior. Interestingly, in habitual high heel wearers, a model for chronically induced calf muscle shortening and declines in fascicle length (Csapo et al., 2010), similar to SCP, larger dynamic fascicles strains have been reported when walking on their toes. This would be analogous to Barber et al. (2017). Corresponding to the current findings, in simulated toe walking, both the soleus and gastrocnemius muscles seem to operate on the ascending limb of their length tension curve (Neptune et al., 2007). Therefore, it could be decisive, where on their individual force-length relationship the fascicles need to operate, which is a potential research goal for a further study.

Noteworthily, the correlation between resting fascicle length and peak push-off power in SCP did not reach significance, which is similar to Martin Lorenzo et al. (2018). However, when relating fascicle length during gait to their resting length, children with SCP that operated at a shorter relative length were able to produce larger ankle power during push-off (**Table 3**). Based on findings of reduced sarcomeres in series in the lower limb muscles of individuals with SCP, e.g., for

the gastrocnemius (Mathewson et al., 2014) and soleus (Mathewson et al., 2014; Mathewson and Lieber, 2015), it can be assumed that the sarcomeres may likely need to operate with little overlap in SCP. Thus, adapting a shorter relative fascicle length during gait, as shown in this study, could be a compensatory strategy to shift the sarcomere operating region on the individual optimal part of the length-tension relationship. Therefore, fascicle and sarcomere lengths should not be equated. To the best of our knowledge, there is no actual experimental data available showing on which part of the length-tension curve the fascicles operate in individuals with SCP during gait. However, experimental instrumented strength tests showed that the maximal plantar flexor force generating capacity of SCP patients is shifted toward plantar flexion angles (Brouwer et al., 1998; Barber et al., 2012; Frisk et al., 2019). Thus, the eccentric fascicle excursions seen in SCP patients most likely reflect an individually excessed demand on weak muscles in unfavorable conditions for active force production. Determining the fascicle force-length relationship experimentally simultaneous with ultrasound during gait, as already performed in healthy individuals (Rubenson et al., 2012; Bohm et al., 2018), could be a promising aim for future studies.

In TD children, a slower fascicle shortening velocity was correlated with a larger push-off power. The dependence of muscle force output on the velocity of the fibers has long been established. During ambulation, fascicles in the plantar flexors usually maintain a relatively low shortening velocity compared with that of the MTU, likely to operate in more favorable regions of the force-velocity curve where muscle efficiency seems to be highest (Lichtwark et al., 2007; Lichtwark and Wilson, 2008). In the past, it could be shown that, as TD children walk faster, ankle power output increases (Schwartz et al., 2008). Worth mentioning, this relationship can also be confirmed when additionally analyzed using the current cross-sectional data ($r = 0.79$, $p < 0.001$). Yet, during faster walking, the force generation ability of the plantar flexors usually decreases, coinciding with an increase in fiber shortening velocity and more activation to generate muscle force (Farris and Sawicki, 2012; Arnold et al., 2013). We suggest that, at the individually preferred walking speed, the behavior of muscles in healthy subjects is likely tuned to minimize consumption of metabolic energy for eliciting contractions. The ability to maintain slower contractile velocities for higher joint power production may thus be a key factor for the velocity healthy humans choose to walk at (Farris and Sawicki, 2012). Accordingly, an inverse relationship between higher SSW speed and lower GM fascicle shortening velocities has been previously also reported in young and old adults (Stenroth et al., 2017). Whether slower fascicle shortening speed may further indirectly reflect Achilles tendon compliance is still subject of scientific controversy (Lichtwark and Wilson, 2008; Arnold et al., 2013; Werkhausen et al., 2019). Interestingly, for children with SCP, there was no significant correlation between fascicle shortening velocity and push-off power in the present study (**Figure 6B**). Since adequate regulation by the central nervous system may be needed to control the operating length and velocity of muscles fascicles during gait by muscle activation (Bohm et al., 2018), we assume that these relationships might be disturbed by poor neural control in SCP patients. However, these aspects were outside the scope of the current analysis.

Limitations

Some limitations need to be considered. First, the current sample is small but homogenous yet the analysis is limited by its cross-sectional design. The results should not be generalized to unilaterally affected children. We admit that solely on the basis of these observed cross-sectional associations in children with bilateral CP, we are unable to legitimately deduce a cause-and-effect relationship, but we provided biomechanical reasoning to explain our findings and also suggest future aspects that need to be explored. From this point of view, this study generates research goals. Analyzing prolonged walking in SCP to study the effect of fatigue and assessing tendon stiffness and fascicle force angle relationships in combination with ultrasound during gait could be aims for future studies.

Second, the pathology in SCP affects several muscles, both from a morphological and coordinative perspective. Thus, further interrelated factors (e.g., neural control) are likely to affect the currently investigated associations to gait kinematics and kinetics. We only focused on the GM muscle, yet other plantar flexors, e.g., the M. soleus, may also play an important role considering its larger volume (Noble et al., 2014) and the fact that the M. soleus contractile behavior was shown to be partly distinctive from the GM in healthy populations (Cronin et al., 2013). Simulations also pointed out that soleus contracture causes more plantar flexion (more equinus) in stance and also aggravates hip and knee flexion (Ong et al., 2019). Future studies may also shift their focus accordingly.

Third, it should be also noted that the gait and related muscle variables were assessed while the study participants walked at a self-selected speed over a level walkway (Kalsi et al., 2016; Barber et al., 2017) or barefoot at a self-selected speed on a treadmill (Hösl et al., 2016). Despite familiarization in both approaches (e.g., to become familiar with walking with the ultrasound probe attached to the leg), walking on a treadmill can affect the children's gait. While no difference in GM muscle fascicle behavior (Cronin and Finni, 2013) as well as in EMG and kinematic patterns (Lorentzen et al., 2018) could be found between treadmill and overground gait in TD adults, some differences may exist for children with SCP: van der Krogt et al. (2015) reported that children with SCP might rely on their hip joint for generating power on treadmills, but how the treadmill may alter the push-off mechanism remains debatable. Albeit not being different between patients and controls, the alterations in walking speed could also have an impact on gait kinetics.

Fourth, the validity of handheld dynamometry for plantar flexor strength measurements may be debatable due to issue in selective motor control and due to the fact that the optimal point on the length-tension curve for active force generation might have potentially been missed in the children with SCP due to the chosen measurement position (ankle joint in neutral). However, despite that shortcoming, correlations could be found

and we showed that muscle weakness might be a key factor for the GM muscle fascicle behavior.

Finally, we chose a resting position in which the knee and ankle joint angles were at 90° and assumed that the gastrocnemius fascicles were off-tension. We opted for this position, which allowed to standardize the angular alignment of the knee and ankle. It was chosen to offload the GM and to ensure that the fascicles were in slack. The gastrocnemius should be considerably off tension due to findings in TD subjects (De Monte et al., 2006) and in subjects with SCP (Park et al., 2019). Investigating healthy individuals, Hoffman et al. (2014) suggested that healthy GM fascicles operate at ~123–135% of their rest length during flat forward walking, which refers to the beginning of the descending limb of their active length-tension curve. In the present study, the GM fascicles of the TD children similarly operated on average at ~114–138% of their seated resting lengths, which supports the validity of our normalizing approach using the seated resting position for measuring resting fascicle length.

SUMMARY AND CONCLUSION

Our results suggest that the gastrocnemius fascicle eccentric contractile behavior may be related to the extent of muscle weakness of patients with SCP. Since we did not find a relationship between the foot landing angle and the eccentric fascicle lengthening behavior, the results of Barber et al. (2017) and Hösl et al. (2016) may not contradict each other. In particular, children with SCP with weaker muscles may therefore be more susceptible to chronic eccentric overloading and pathological remodeling processes, which has to be clarified in future studies. Carefully speaking, the correlations also suggest that atrophy of gastrocnemius MT may be related to reduced plantar flexion and may promote undesired knee flexion during gait. Therefore, sole calculations of musculoskeletal models showing shorter gastrocnemius MTU lengths could be misleading in treatment decision-making. In addition, we provided evidence that a thicker gastrocnemius muscle belly and particularly longer gastrocnemius fascicles could be reasonable morphometric properties that should be targeted in interventions for pediatric and juvenile patients with SCP, since they may relate to a more upright and faster gait pattern. Despite longer fascicles are *per se* favorable for children with SCP, we found that those patients who managed to operate on shorter relative length with respect to their resting length produced a better push-off. The peak push-off power in individuals with SCP is likely affected by the fascicle force-length relationship (probably altered sarcomere length and number), while in TD individuals the force-velocity relationship could instead be decisive.

DATA AVAILABILITY STATEMENT

Data supporting the major conclusions of this manuscript can be made available by the authors, upon reasonable requests.

ETHICS STATEMENT

The studies involving human participants and were reviewed and approved by ethics committee at Technical University Munich Rechts der Isar. Written informed consent to participate in this study was provided by the participants and their legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AK and MH developed the idea, planned and designed the data reanalysis, and prepared the current manuscript. MH, AA, HB, and AZ planned and conducted the initial experimental protocol. MH analyzed the data, and AZ assisted in data collection and analysis during her Master's Degree at the TU Munich. All authors contributed to both the interpretation and discussion of the results and critically revised and edited the manuscript. All authors read and approved the submitted version.

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Monitoring Muscle-Tendon Adaptation Over Several Years of Athletic Training and Competition in Elite Track and Field Jumpers

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Differences in muscle and tendon responsiveness to mechanical stimuli and time courses of adaptive changes may disrupt the interaction of the musculotendinous unit (MTU), increasing the risk for overuse injuries. We monitored training-induced alterations in muscle and tendon biomechanical properties in elite jumpers over 4 years of athletic training to detect potential non-synchronized adaptations within the triceps surae MTU. A combined cross-sectional and longitudinal investigation over 4 years was conducted by analyzing triceps surae MTU mechanical properties in both legs via dynamometry and ultrasonography in 67 elite track and field jumpers and 24 age-matched controls. Fluctuations in muscle and tendon adaptive changes over time were quantified by calculating individual residuals. The cosine similarity of the relative changes of muscle strength and tendon stiffness between sessions served as a measure of uniformity of adaptive changes. Our cross-sectional study was unable to detect clear non-concurrent differences in muscle strength and tendon stiffness in elite jumpers. However, when considering the longitudinal data over several years of training most of the jumpers demonstrated greater fluctuations in muscle strength and tendon stiffness and hence tendon strain compared to controls, irrespective of training period (preparation vs. competition). Moreover, two monitored athletes with chronic Achilles tendinopathy showed in their affected limb lower uniformity in MTU adaptation as well as higher fluctuations in tendon strain over time. Habitual mechanical loading can affect the MTU uniformity in elite jumpers, leading to increased mechanical demand on the tendon over an athletic season and potentially increased risk for overuse injuries.

Keywords: Achilles tendon, triceps surae, time course, imbalance, athletic training, tendinopathy

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INTRODUCTION

Tendons are an integral part of musculotendinous units (MTUs) and their primary function is to transmit forces from muscles to rigid bone levers to produce joint motion. As a part of their viscoelastic nature, elasticity of the tendons can enhance force and power generating capacity of MTUs by storing and releasing strain energy and by allowing muscles to operate under more favorable conditions related to the force-length-velocity relationship (Biewener and Roberts, 2000; Hof et al., 2002). Thus the mechanical properties of tendons need to be considered as important

determinants of musculoskeletal function, and it is not surprising that they have been shown to affect athletic performance (Bojsen-Møller et al., 2005; Stafilidis and Arampatzis, 2007). Not only are tendons vitally important for movement and sports performance, movement and exercise are also essential for tendons. As living tissue, tendons respond to mechanical loading by changing their metabolism, which can lead to alterations in their material composition (e.g., changes in extra-cellular matrix or cross-linking) and their morphological properties (cross-sectional area) with the latter as a more long-term response (Bohm et al., 2015a; Wiesinger et al., 2015). Accordingly, when a MTU is constantly experiencing increased mechanical loading (e.g., by resistance training) it is generally observed that the associated adaptive enhancements in muscle strength lead also to a markedly greater tendon stiffness (Kubo et al., 2001, 2012; Arampatzis et al., 2007a,b; Bohm et al., 2014). The reported modifications in tendon stiffness may exhibit a protective homeostatic process to withstand greater functional demand due to muscle strength gains. Nevertheless, the muscle strength gains seem to be merely in a moderate association with modifications in tendon mechanical properties (Arampatzis et al., 2007a,b). This may be explained by discrepancies between muscle and tendon in their adaptive response to the experienced mechanical stimuli (Arampatzis et al., 2007a) and in the timescale of adaptation (Kubo et al., 2012) with distinctively slower response rates for tendons due to lower tissue turnover (Heinemeier et al., 2013). Hence, an imbalance between the generated muscle forces and the strength of the tendon may occur over the course of training process, placing the tendon under greater mechanical demand (i.e., higher strain).

Compared to tendons, muscles seem to possess superior adaptability in reaction to a broad variety of exercise modalities (Moss et al., 1997), the former responding most effectively to mechanical loads creating high magnitudes of tendon strain over extended time durations (Arampatzis et al., 2007a; Bohm et al., 2014). These adaptive discrepancies within the MTU could become crucial for athletes constantly involved with high mechanical loading profiles in their training process, particularly in sport disciplines with frequent plyometric loading, such as track and field sprinters and jumpers (Stefanyshyn and Nigg, 1997, 1998; Dorn et al., 2012; Willwacher et al., 2017). By its nature plyometric training is characterized by high magnitude mechanical loading, though the time spans over which those high forces are applied tend to be rather short. This might be a reason why various plyometric loading regimens do not always lead to clear adaptive increases in tendon stiffness along with muscle strength gains (Kubo et al., 2007; Fouré et al., 2010; Houghton et al., 2013; Bohm et al., 2014; Hirayama et al., 2017). From a biomechanical viewpoint, muscle strength improvements with insufficient compensatory adaptations in tendon mechanical properties would potentially increase the mechanical demand for the tendon (i.e., higher strain). In fact, it has recently been demonstrated that habitual athletic training that consists mainly of plyometric loading affects the uniformity of muscle and tendon adaptation in adolescents (Mersmann et al., 2016) with potential implications for injury and tendinopathy (Wren et al., 2003; Fredberg and Stengaard-Pedersen, 2008). The fact that athletic training for elite athletes consists of complex loading variations and includes different training modalities (Haugen et al., 2019) could lead to considerable fluctuations in muscle strength in the intermediate term, which may have implications not only for the performance but also for the integrity of the tendon. Regarding that a clear pathogenesis of the development of tendon overuse is still unknown, the above mentioned provides a more mechanistic explanation along other more physiological concepts behind pathological changes in tendon (e.g., immunoregulation/excessive immune response).

The Achilles tendon (AT) is susceptible to injury potentially due its noticeably low safety factor (the ratio of ultimate and operational stress) when compared with other tendons (Ker et al., 1988; Magnusson et al., 2001). Accordingly it does not seem so unexpected that there is a high prevalence of AT tendinopathies in adult athletes (Janssen et al., 2018). This supports the assumption that periods of asynchronous muscle-tendon adaptation in elite jumping athletes might be of clinical relevance and that further research is needed to explore whether athletic training is an influential factor in non-uniform adaptation between muscle and tendon. To our knowledge the time course of muscle and tendon adaptations in adult athletes over several years of athletic training and competition is unknown.

The aim of the current investigation was to regularly monitor training-induced alterations in triceps surae (TS) muscle strength, tendon stiffness and tendon strain in the legs of elite jumpers over several years of training in order to detect potential non-uniformities within the TS MTU due to habitual mechanical loading. Potential alterations in TS MTU properties in elite athletes were compared with the variations in a control group of healthy recreationally active adults of similar age range. On the basis that elite athletes are subjected to high variation in mechanical loading profiles over a season, we hypothesized that elite athletes experience greater fluctuations in MTU mechanical properties over time and demonstrate temporary increases in mechanical demand on the tendon as a result of non-uniform MTU adaptation. For this reason we conducted a combined cross-sectional and longitudinal investigation by analyzing a total of 67 healthy young elite jump track and field athletes competing at international level, from which a sub-group of 18 elite jumpers were monitored regularly over 1 year of training. As part of this long-term investigation six of the elite athletes were measured regularly over a 4-year period to investigate possible differences between the preparation and competition periods. Two additional elite athletes with unilateral AT tendinopathy and one athlete following unilateral AT reconstruction will be discussed separately.

MATERIALS AND METHODS

Participants and Experimental Design

In this investigation on TS MTU adaptation we were able to voluntarily recruit 67 healthy young elite international level jumping event track and field athletes (35 male and 32 female) from the German national team (age: 23 ± 4 years; body mass: 73 ± 11 kg; body height: 183 ± 9 cm; mean and

standard deviation; for further description and personal records see **Supplementary Material**). Additionally, 24 young healthy recreationally active (12 male and 12 female; age: 24 ± 3 years; body mass: 72 ± 12 kg; body height: 177 ± 10 cm) acted as control group. Excluded were athletes with prior AT injuries (ruptures, tendinopathy etc.) during the preceding six months, as it may have possibly affected the findings. Accordingly in total 91 subjects were included in our cross-sectional investigation. Next to the healthy participants, three additional athletes with current AT injuries (two athletes with chronic tendinopathy and one with a recent tendon rupture) voluntarily participated in the study and were separately included to the study. The study was ethically approved by the local committee of the German Sport University Cologne and all participants provided their written informed consent prior to the study in accordance to the recommendations in the Declaration of Helsinki.

As a part of the day-to-day training routine at their corresponding Olympic training centers or training camps (**Figure 1A**), the TS MTU biomechanical properties (maximal ankle plantar flexion moment, AT stiffness and maximal tendon strain) were assessed in both legs of all participating athletes, concurrently or precisely prior to the competition period (cross-sectional investigation). In order to distinguish between the limbs, the preferred jumping leg was considered as the take-off leg and the contralateral leg as the swing leg. The hop leg in triple jumpers was defined as the take-off leg. Similar to the athletes, measurements with the control group were performed on both limbs in the cross-sectional investigation (take-off leg defined via a questionnaire as the preferred jumping leg), whereas in the longitudinal investigation only the take-off leg was analyzed. In the injured athletes, the comparison was made between the healthy and affected (injured) limb.

Longitudinal Investigation Over 1 Year

Due to the a lack of appropriate longitudinal data of MTU adaptive changes in elite track and field athletes a power analysis for a target sample size was not possible, therefore it was aimed to recruit as many participants as possible. Out of the 91 healthy participants from our cross-sectional investigation, we were able to recruit 40 elite athletes and 15 controls to participate over a minimum of 1 year observation period. However, only 18 athletes and 12 controls were considered as valid for our longitudinal investigation over 1 year by reaching our criteria for the statistical analysis (set criteria: regular measurement about every three- to five-weeks) while the rest dropped out due to missing time, various injuries (including lower limb MTU injuries) or due to other reasons. Next to the 18 monitored healthy athletes we were able to recruit and regularly measure two elite athletes with a chronic unilateral AT tendinopathy and one elite jumping athlete 10 months after AT rupture and reconstruction monitored over a follow up time period of 2 years. The data of these three athletes with AT injury were not included in the statistical analysis of the pool of healthy elite athletes and were considered separately. For the statistical analysis of the elite athletes ($n = 21$), measurements were conducted on both limbs at every three- to five-weeks over the 1 year observation window (only for the elite athlete with AT rupture less frequent measurements were conducted). For the control group only the take-off leg was considered, as previous studies have shown similar TS MTU mechanical properties between limbs in healthy adults (Bohm et al., 2015b) and thereby to reduce the amount of measurements taken.

Longitudinal Investigation Over 4 Years

Twelve jumpers from the initial pool of healthy elite athletes agreed to participate over an extended 4 year time period of TS MTU monitoring. However, only in six elite athletes it was possible to determine the bilateral MTU biomechanical properties about every three- to five-weeks over the 4 years period, therefore all other athletes were excluded from further analysis (dropped out due to missing time, injury or other reasons). Those regularly monitored six elite athletes served for the investigation of differences between the preparation and competition periods in bilateral MTU adaptive changes over 4 years.

Analysis of Muscle and Tendon Mechanical Properties

The TS MTU biomechanical properties (maximal ankle plantar flexion moment, AT stiffness and maximal AT strain) were assessed in all participants on a custom-made device (**Figure 1B**) using synchronous ultrasonography and dynamometry as in our previous study (Epro et al., 2019). Briefly, the participants were positioned with their knee and ankle joints fixed at 90° (with their foot and thigh perpendicular to the shank) and their foot set on a strain-gauge type dynamometer (1000Hz; TEMULAB®, Protendon GmbH & Co. KG, Aachen, Germany; **Figure 1B**). The foot was set on the dynamometer (**Figure 1B**) with the midpoint of the malleolus lateralis aligned with the dynamometer's axis of rotation (Ackermans et al., 2016). A laser-guided electrical potentiometer system measuring the linear displacement was used to position each participant in a standardized manner, which allowed recreating the exact position for each individual at any measurement time point. Prior to each measurement, all participants performed an individual warm-up, which was succeeded by a standardized visually guided warm-up in the software consisting of several submaximal and three maximal isometric contractions to "precondition" the tendon (Maganaris, 2003).

Subsequently, the maximal ankle plantar flexion moment as well as the force–elongation relationship of the tendon during the loading phase were assessed by performing isometric ankle plantar flexion contractions at different joint moment levels: three verbally encouraged maximal voluntary ankle plantar flexion contractions (MVC), succeeded by three visually guided sustained contractions at 30, 50, and 80% of the maximal joint moment obtained in the prior MVCs. The resultant joint moments acting about the ankle joint were calculated using inverse dynamics and a preceding passive measurement (relaxed muscles in the fixed position) was performed to account for the gravitational moments. The alignment of the axes of rotation of the ankle and the dynamometer (**Figure 1B**) allowed to consider the ankle joint moment to be quasi-equal with the acquired moment of the force plate (Ackermans et al., 2016). Nevertheless,

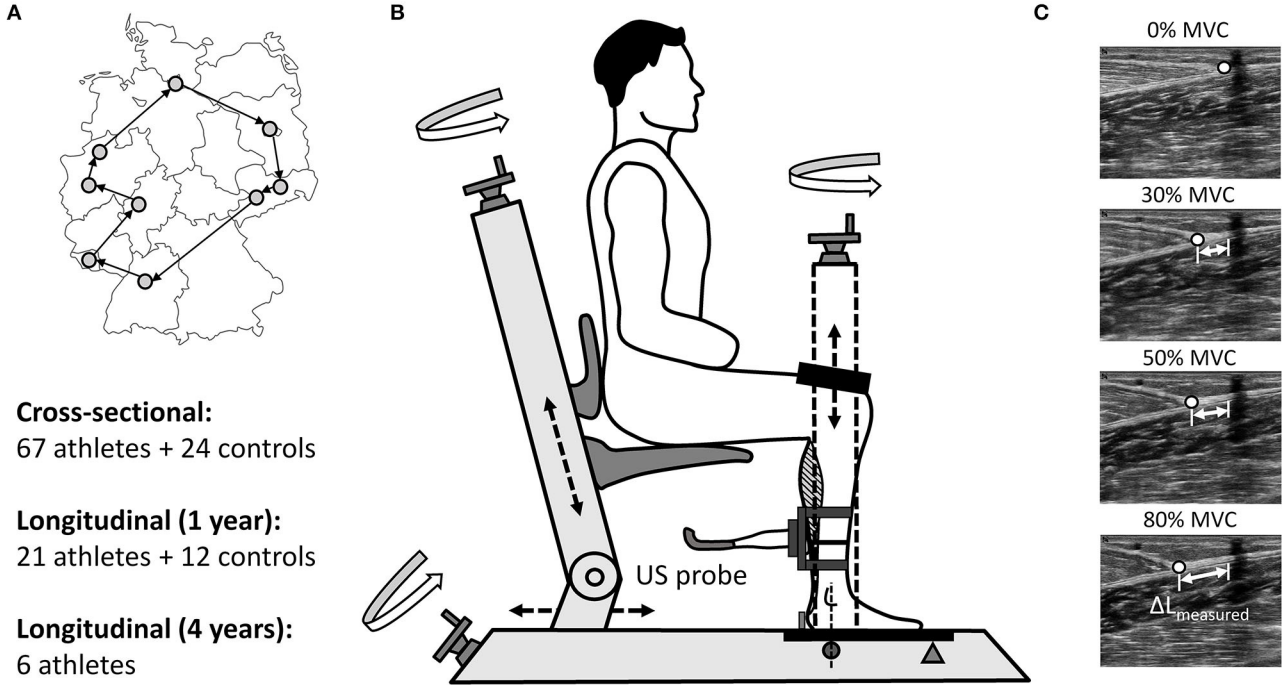

FIGURE 1 | Measurement locations at the Olympic training centers in Germany incl. study design **(A)** and the used custom-made analysis device (TEMULAB®, Protendon GmbH & Co. KG, Aachen, Germany) to assess TS MTU mechanical properties incorporating dynamometry and ultrasonography **(B)**. In order to determine the force-elongation relationship of the tendon the displacement of the myotendinous junction (MTJ) of the m. gastrocnemius medialis ($\Delta L_{measured}$) was digitized at rest (0%) and at three sustained contractions (target joint moment constantly held for 3s) at fixed target ankle joint moment levels (30, 50, and 80%; **C**).

it needs to be noted, that the resultant ankle plantar flexion moment is an approximation of the net moment generated by the TS muscle, as the current setup did not account for the individual moment contributions of the synergistic agonist muscles and the antagonist dorsiflexors. Subsequently, the resultant ankle joint moment was further used to calculate the acting AT force by dividing it by the tendon moment arm acquired from a previous study (Maganaris et al., 1998). In order to exclude the effect of any potential changes in body mass on the calculations of fluctuations in muscle strength over time, we used the absolute maximal joint moments (Nm) instead of body mass normalized moments.

After tendon preconditioning, the AT resting length was measured using the integrated laser-guided electrical potentiometer system as the distance from the myotendinous junction (MTJ) of the m. gastrocnemius medialis to the most proximal point of the tuber calcanei (both defined and marked beforehand using ultrasonography). Similar to the positioning of each participant on the dynamometer, the laser-guided electrical potentiometer system allowed maintaining the probe position constant for each subject and leg during all measurements. During the following plantar flexion contractions the movement of the MTJ of the m. gastrocnemius medialis was analyzed with a firmly fixed 40 mm linear array ultrasound probe (27 Hz; MyLab^{TM}One, Esaote; Genoa, Italy) and manual digitization using a custom-made software in Python (ver. 2.7.0; **Figure 1C**). The ultrasound probe was secured above the MTJ on the shank using a casing with adjustable straps to avoid movement in relation to the skin. The MTJ displacement was traced at the resting state (0% MVC) and in the following three sustained contractions (steadily held target joint moment for 3 s) at the target ankle joint moments (30, 50, 80% MVC; **Figure 1C**). The participants had to repeat the specific trial, when they were unsuccessful to hold a steady state for 3s within a range of ±5% of the target joint moment. A linear extrapolation of the elongation at 50 and 80% target joint moments was used to estimate the tendon elongation at maximal ankle joint moment (100%) as in Ackermans et al. (2016), because specific strict instructions about loading rate and maintaining the generated moments at certain given level may limit the ability to contract maximally. Moreover, this approach to calculate maximal tendon strain can be considered as a valid measure as tendon elongation between 50 and 100% of the MVC can be assumed to be rather linear and because a considerable amount of the net elongation occurs within the first 25% of the MVC (McCrum et al., 2018). The effect of the inevitable ankle joint angular rotation on the measured tendon elongation (Muramatsu et al., 2001) was taken into account during each contraction by subtracting the elongation caused by ankle joint changes. Ankle joint angle during each contraction was obtained through the inverse tangent of the ratio between the heel lift (measured via a heel-potentiometer) and the distance from the ankle joint axis to the fifth metatarsal bone (Ackermans et al., 2016). Subsequently, the ratio between the change in the calculated tendon force and the resultant tendon elongation between 30 and 80% of maximum tendon force was

used to estimate the AT stiffness. Before conducting the current study we performed several pilot studies to test the accuracy and validity of the implemented method to assess the TS MTU mechanical properties. The main findings of these pilot studies are provided in the **Supplementary Material** under the chapter "Methodological considerations and pilot data."

Statistics

The normality of distribution and homogeneity of variance of the data was confirmed using the Shapiro-Wilk and Levene's test. For the cross-sectional analysis, a two-way repeated measures analysis of variance (ANOVA) was performed with the group (athletes vs. control) and leg (take-off vs. swing leg) as factors to investigate potential differences in TS muscle strength, AT stiffness and maximal AT strain. For the longitudinal investigations, firstly the uniformity of muscle and tendon adaptive changes over the 1 year time period in both elite athletes and control subjects were analyzed using individual absolute residuals (averaged over all measurement sessions) from a general linear model as a measure of fluctuations in TS MTU properties and secondly by forming cosine similarity (CS) of the relative changes between the individual measurement sessions (Mersmann et al., 2016):

$$CS = \cos(\theta) = \frac{X \cdot Y}{\|X\| \cdot \|Y\|} = \frac{\sum_{i=1}^{n-1} X_i Y_i}{\sqrt{\sum_{i=1}^{n-1} X_i^2}\sqrt{\sum_{i=1}^{n-1} Y_i^2}}$$

X and Y denote the vectors of the relative changes between sessions ($i = 1,\ldots, n$) and n refers to the individual total number of measurements. CS values range between 1 (illustrating equal direction of the vectors, i.e., high adaptation similarity) to−1 (opposing direction, i.e., low adaptation similarity). For the 4 years investigation (eight consecutive seasons), the residuals and CS were averaged separately for the preparation (mid-March to mid-May and start of October to end of December) and competition periods (start of January to mid-March and mid of May to mid-September). A one-way ANOVA was implemented to investigate potential subject group-related differences (take-off leg vs. swing leg of athletes vs. take-off leg controls) in the fluctuations (residuals) and CS of muscle and tendon biomechanical properties over the 1 year time period. Potential training period (preparation vs. competition period) influence in the fluctuations and CS in MTU adaptation over 4 years of athletic training was examined using a two-way ANOVA with leg and training period as factors. In the case of a detected significant main effect or interaction a Bonferroni *post hoc* comparison was performed. In addition, in order to evaluate the strength of potential training-induced alterations in cross-sectional and longitudinal investigations the partial eta squared (η_p^2) was calculated (Cohen, 2013). Potential subject group differences in age, body mass and body height between elite athletes and controls were compared using an independent samples *t*-test. All statistical calculations and analyses were performed using custom-made MATLAB scripts (version 2020b; The MathWorks, Natick, MA, USA) or SPSS statistics software (version 26.0; IBM, Armonk, NY, USA) with the results in the text and figures provided either as means and standard deviation (SD) or as boxplots (median and interquartile range between 25th and 75th percentile along with minimum and maximum values) and the level of significance set at $\alpha = 0.05$.

RESULTS

Cross-Sectional Investigation: Elite Athletes vs. Controls

For the cross-sectional investigation 67 healthy elite jumpers (35 male and 32 female) and 24 recreationally active young adults (12 male and 12 female; control group) were considered for statistical analysis with respect to potential leg and subject group effects on MTU properties. Irrespective of the analyzed limb (take-off vs. swing), elite jumpers demonstrated in comparison to the control group significantly higher TS muscle strength (average values of both legs: athletes 303 ± 85 vs. controls 241 ± 65 Nm; subject group effect: $F_{(1, 89)} = 10.415$, $P = 0.002$, $\eta_p^2 = 0.105$) as well as greater tendon stiffness (653 ± 187 vs. 570 ± 131 N/mm; $F_{(1, 89)} = 4.004$, $P = 0.048$, $\eta_p^2 = 0.043$) with similar relative difference between groups (∼20% in TS muscle strength vs. ∼15% in tendon stiffness). Hence, maximal tendon strain was not significantly different between groups independent of the analyzed leg (average values of both legs: athletes 4.8 ± 1.1 vs. controls 4.8 ± 1.0%). Concerning the between leg analysis, we found limb-specific differences in TS MTU mechanical properties in jumpers but not in controls. Elite jumpers showed significantly higher muscle strength (310 ± 89 vs. 296 ± 84 Nm, $P = 0.001$, $\eta_p^2 = 0.127$) and tendon stiffness (675 ± 195 vs. 630 ± 186 N/mm, $P < 0.001$, $\eta_p^2 = 0.226$) in their take-off in comparison to their swing leg. No significant leg-specific differences were detected for maximal AT strain in the elite jumpers (take-off leg 4.6 ± 1.0 vs. swing leg 4.8 ± 1.1%). A significant group-difference between the elite jumpers and control group was found for the body height (183 ± 9 vs. 177 ± 10 cm for elite jumpers and control respectively, $P = 0.008$), but not for age (23 ± 4 vs. 24 ± 3 years) and body mass (73 ± 11 vs. 72 ± 12 kg).

TS MTU Adaptive Changes Over 1 Year: Elite Athletes vs. Controls

Eighteen healthy athletes (8 males and 10 females) were considered as valid for our longitudinal investigation over 1 year as they reached our criteria for the longitudinal statistical analysis (set criteria: measurement about every three- to five-weeks). When considering all data points analyzed within the 12 months observation period we found greater fluctuations of muscle strength over time in elite jumpers compared with controls irrespective of the analyzed leg (i.e., greater residuals; take-off leg of athletes vs. take-off leg control $P = 0.010$ and swing leg of athletes vs. take-off leg control $P = 0.049$; **Figure 2**). Furthermore, although not reaching statistical significance, there were tendencies for greater fluctuation in tendon stiffness ($P = 0.074$; **Figure 2**) and in maximal tendon strain for elite athletes take-off leg in comparison to control ($P = 0.092$; **Figure 2**). In addition, for the CS a main effect was detected ($P = 0.030$), with the athletes take-off leg in comparison to the

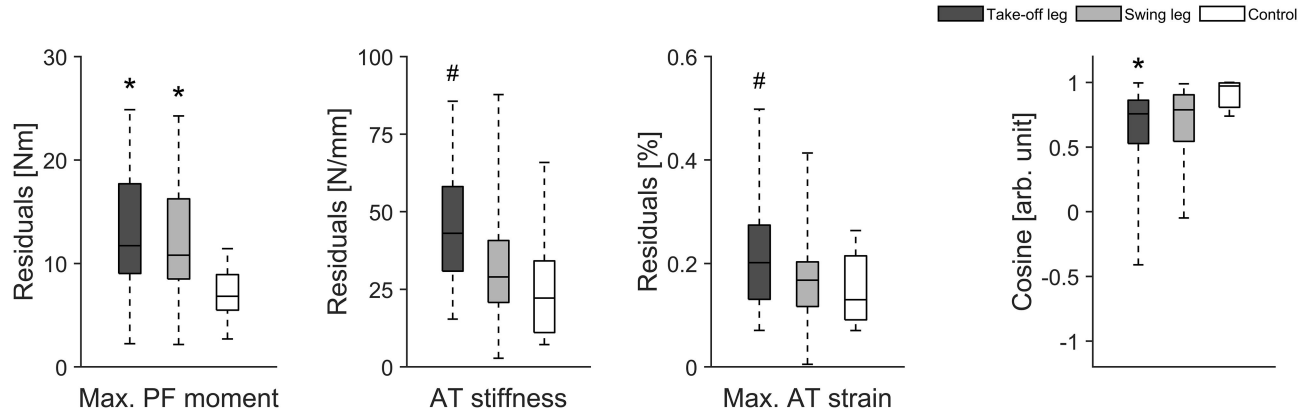

FIGURE 2 | Box plots of muscle strength (Max. PF moment), tendon stiffness (AT stiffness) and maximal tendon strain (Max. AT strain) as well as the cosine similarity (Cosine) of the relative changes of muscle strength and tendon stiffness between sessions over the 1 year observation period in 18 healthy elite jumpers (take-off and swing leg) and 12 healthy control subjects (take-off leg). *Statistically significant difference to control ($P < 0.05$). #Tendency for significant difference to control ($0.05 < p < 0.1$).

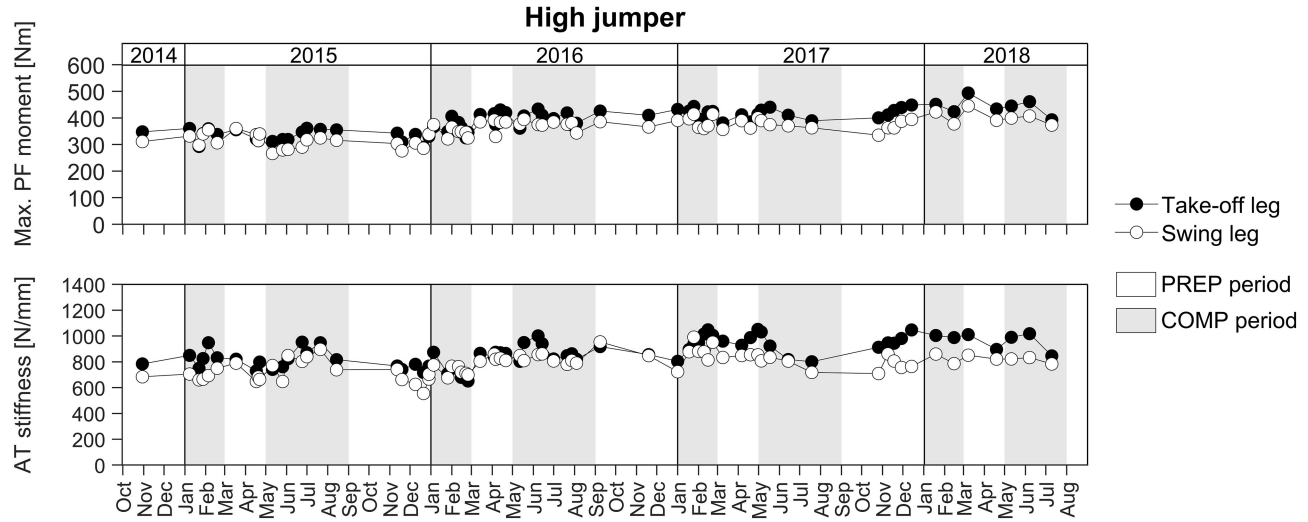

FIGURE 3 | Adaptive changes in TS muscle strength (Max. PF moment) and tendon stiffness (AT stiffness) in the take-off and swing leg of one elite high jumper over a period of 4 years of athletic training separated into preparation (PREP) and competition (COMP) periods.

control group demonstrated statistically significant ($P = 0.026$) lower similarities (lower CS) in their adaptive changes of muscle strength and tendon stiffness (**Figure 2**).

TS MTU Adaptive Changes Over 4 Years: Preparation vs. Competition Period

For the longitudinal investigation over 4 years we were able to analyse six healthy male elite athletes on a regular basis (about every three- to five-weeks) over eight preparation and eight competition periods. Exemplarily **Figure 3** represents the changes in TS muscle strength and tendon stiffness in both legs of one elite high jumper over a period of 4 years of athletic training separated into preparation and competition periods. The total number of measurements for those six elite athletes over the 4 years period ranged from 36 to 73 with 51% measurements in the preparation and 49% measurements in the competition period. Independent of the analyzed leg, there were no significant differences in fluctuations of muscle strength, tendon stiffness and maximal tendon strain over time (no differences in the residuals) between preparation and competition period (**Figure 4**). Further, there were no significant differences in CS in the adaptive changes of muscle strength and tendon stiffness for the preparation in comparison to the competition period (**Figure 4**).

TS MTU Adaptive Changes in Elite Athletes With AT Injury

In addition to the pool of 18 healthy athletes we were able to monitor two elite athletes with a unilateral AT tendinopathy and one elite athlete following a unilateral AT reconstruction over several years of athletic training. At baseline, the two elite athletes with a unilateral AT tendinopathy showed lower

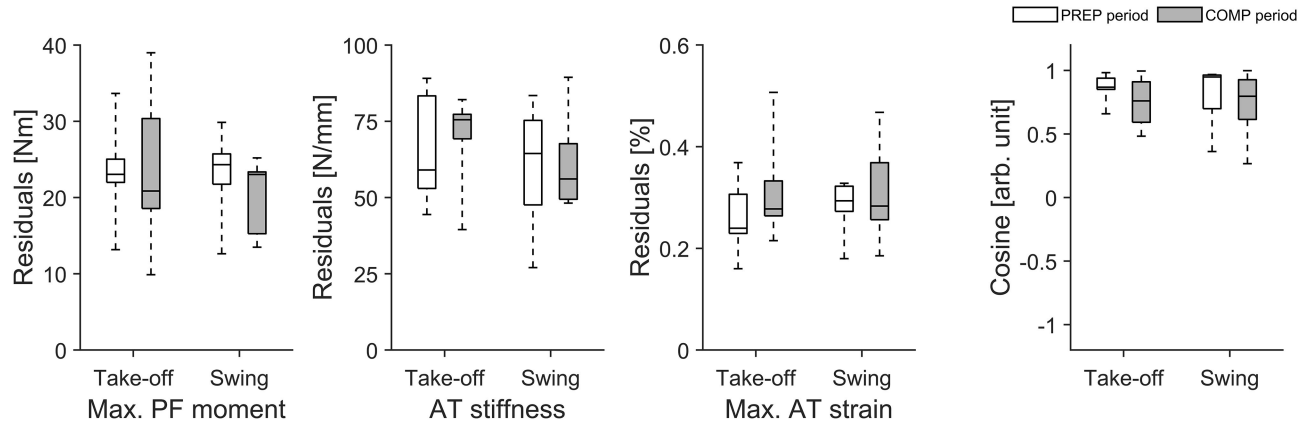

FIGURE 4 | Box plots of residuals in muscle strength (Max. PF moment), tendon stiffness (AT stiffness) and maximal tendon strain (Max. AT strain) as well as in cosine similarity (Cosine) of the relative changes of muscle strength and tendon stiffness between sessions over 4 years during preparation (PREP) and competition (COMP) period in six elite jumpers (take-off and swing leg).

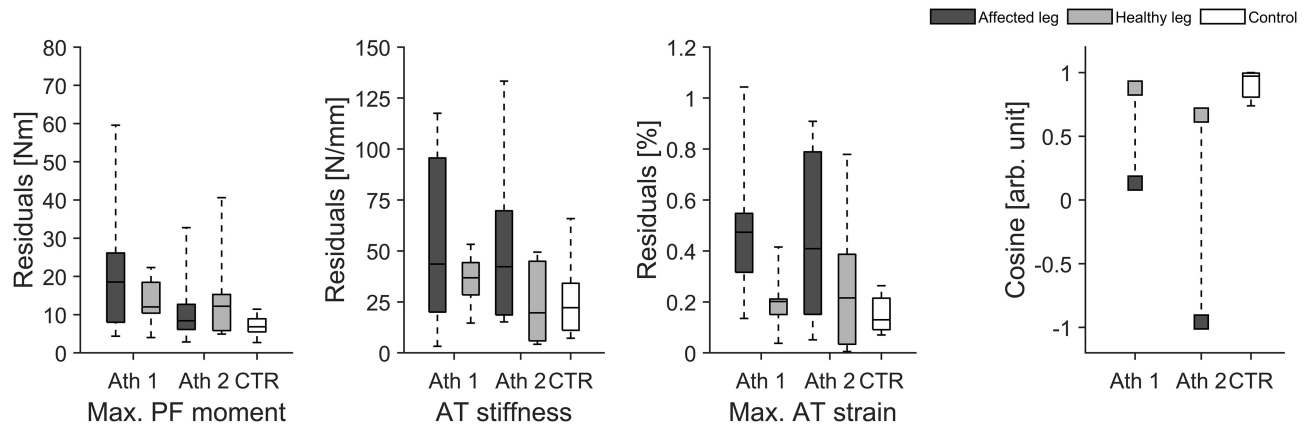

FIGURE 5 | Box plots of residuals in muscle strength (Max. PF moment), tendon stiffness (AT stiffness) and maximal tendon strain (Max. AT strain) as well as the cosine similarity (Cosine) of the relative changes of muscle strength and tendon stiffness between sessions over the 1 year observation period for the affected and healthy leg in two elite jumpers (Ath 1 and Ath 2) with chronic AT tendinopathy and in the control group (CTR; take-off leg; $n = 12$).

tendon stiffness in their affected (injured) limb compared to their unaffected limb (average values for both athletes: affected limb 564 ± 127 N/mm vs. healthy limb 690 ± 242 N/mm) with similar muscle strength values between limbs (303 ± 61 Nm vs. 312 ± 59 Nm). As a consequence, maximal tendon strain was for both athletes higher in their affected limb (5.3 ± 0.1 vs. 4.6 ± 0.2%). We were able to monitor these two elite athletes over a period of 1 year of athletic training and the two athletes with AT tendinopathy showed in their affected limb a lower uniformity (i.e., lower CS) in the adaptive changes of muscle strength and tendon stiffness in comparison to the healthy limb and healthy control group (**Figure 5**). Furthermore, the affected limb showed greater fluctuations (higher residuals) in muscle strength as well as in tendon stiffness and as a consequence in tendon strain over time in comparison to their healthy limb and healthy controls (**Figure 5**).

The remaining injured elite jumper was measured 10 months after an Achilles tendon rupture and reconstruction and we were able to monitor the jumper's bilateral MTU adaptive changes over a follow up time period of 2 years. The data in **Figure 6** indicates that the affected leg shows on average lower muscle strength (∼57%) but about 15% higher Achilles tendon stiffness (with more than 60% lower maximal strain) in comparison to the healthy leg despite over 2.5 years of intense rehabilitation and athletics training.

DISCUSSION

Muscles and tendons both adapt to mechanical loading. However, differences in their responsiveness to mechanical stimuli and the rates of adaptive changes may disrupt the fine-tuned interaction of the MTU, increasing the risk for overuse injuries. In the current work we monitored training-induced alterations in muscle strength and tendon stiffness in young elite jumpers over 4 years of athletic training in order to detect potential non-uniformities within the TS MTU due to habitual

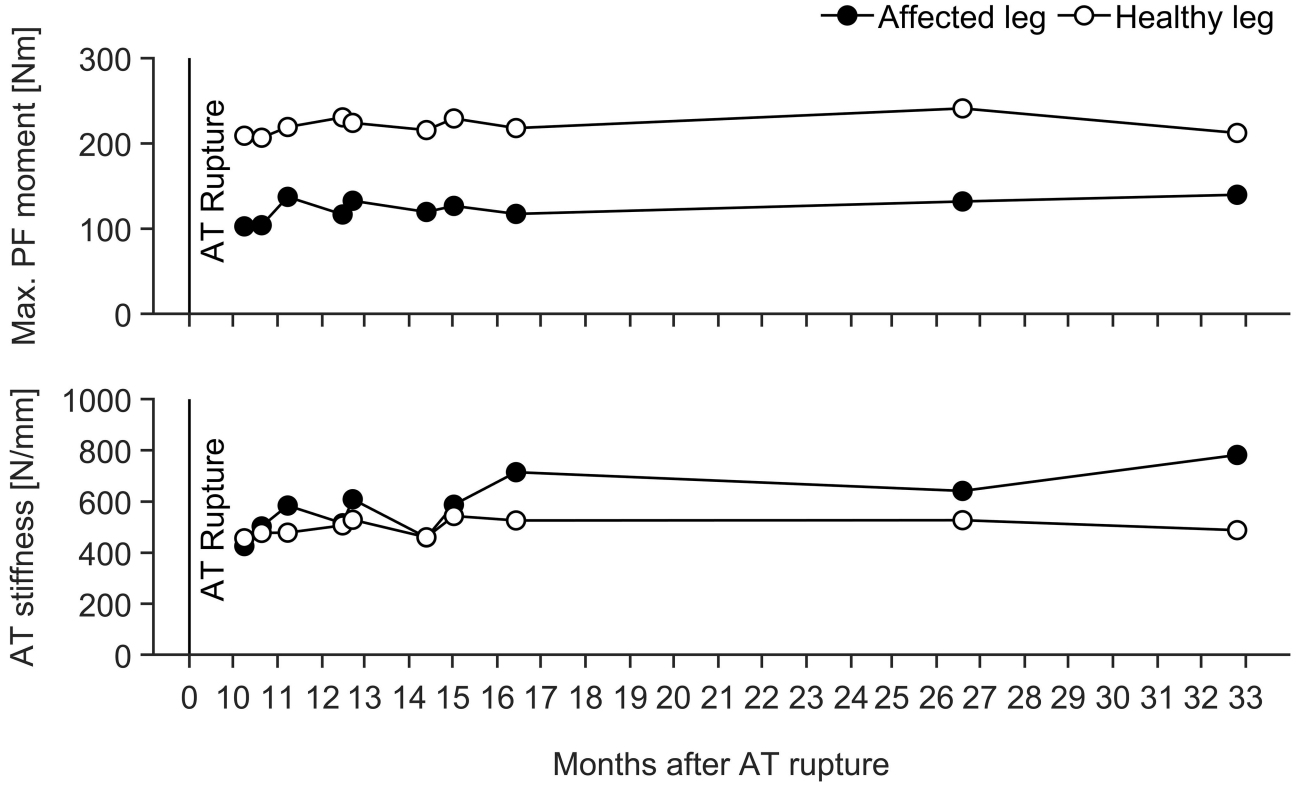

FIGURE 6 | Adaptive changes in TS muscle strength (Max. PF moment) and tendon stiffness (AT stiffness) in the affected and healthy leg of an elite athlete with an Achilles tendon rupture and reconstruction during a follow up time period of 2 years. Maximal tendon strain ranged from 1.5 to 2.5% and from 3.9 to 4.9% for the affected and healthy leg over the entire observation window, respectively.

mechanical loading. When considering the longitudinal data over several years of athletic training most of the jumpers demonstrated greater fluctuations in TS muscle strength and tendon stiffness (and hence tendon strain) compared to controls, irrespective of training period (preparation vs. competition). Moreover, two elite athletes with chronic AT tendinopathy showed lower uniformity (lower CS) in their affected leg in the adaptive changes of muscle strength and tendon stiffness and higher fluctuations in tendon strain. These results support our hypothesis that habitual mechanical loading can affect the uniformity within the MTU in elite jumpers, suggesting a temporarily increased mechanical demand on the tendon over a season and potentially higher injury risk.

Although most resistance exercise interventions implementing high tendon strain magnitudes are regularly reporting comparable changes in tendon and muscle biomechanical properties (Kubo et al., 2001; Arampatzis et al., 2007a), purely plyometric exercise interventions show tendon adaptations that are not concurrent with those of skeletal muscle (Kubo et al., 2007, 2017; Fouré et al., 2010; Sáez-Sáez de Villarreal et al., 2010; Houghton et al., 2013; Bohm et al., 2014; Hirayama et al., 2017). A feasible explanation for this may be that tendons adapt less effectively to loading profiles characterized by short tendon strain durations (Bohm et al., 2014)—as in plyometric activities such as jumping or sprint running. Yet, our cross-sectional investigation comprising a total of 91 subjects did not detect clear non-concurrent increases in muscle strength and tendon stiffness in elite jumpers. Irrespective of the analyzed leg, differences were detected for both TS muscle strength and tendon stiffness ($0.043 \leq \eta_p^2 \leq 0.105$), and hence similar levels of strain during maximum contractions between athletes and controls were observed (on average 4.8% in both the athlete and the control group). Given that tendon strain was not significantly greater in athletes compared to controls, one might suggest that repetitive plyometric and habitual mechanical loading during training seems not to provoke any measurable non-uniform adaptation within the TS MTU in elite jumpers. Hence, it is reasonable to suggest that the reported alterations in tendon stiffness in elite athletes may present a protective mechanism to maintain the tendon integrity in response to the increased demand due to the changed muscular strength.

As for most previous investigations on MTU structure and function in adult athletes (Kongsgaard et al., 2005; Arampatzis et al., 2007b; Stafilidis and Arampatzis, 2007; Wang et al., 2012; Couppé et al., 2013; Epro et al., 2019), the above findings rely on a single time point (data were assessed directly prior to the competition period) and therefore potential fluctuations in muscle strength and tendon stiffness over a period of athletic

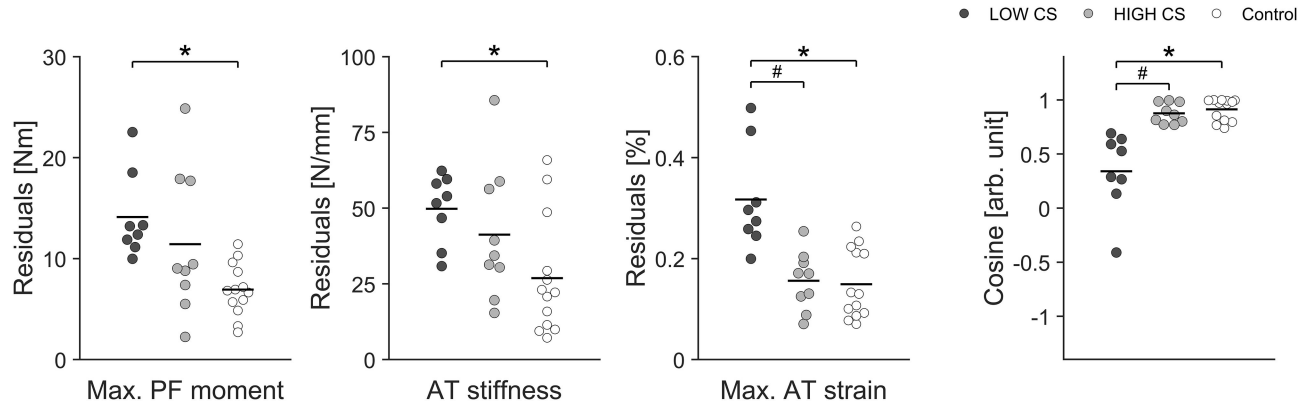

FIGURE 7 | Individual residuals and group means (dark line) in muscle strength (Max. PF moment), tendon stiffness (AT stiffness) and maximal tendon strain (Max. AT strain) as well as the cosine similarity (Cosine) of the relative changes of muscle strength and tendon stiffness between sessions over the 1 year observation in the take-off leg of the Low CS and High CS group of elite jumpers and in the control group. *Statistically significant difference between Low CS and control group ($P < 0.05$). #Statistically significant difference between Low CS and High CS group ($P < 0.05$).

training is unknown. As part of this work on muscle and tendon adaptation, we investigated for the first time MTU biomechanical properties at about three- to five-week intervals over several years of athletic training and competition. When all data points analyzed within the 12-month observation period were taken into consideration (for the 18 healthy athletes monitored), we found greater fluctuations of muscle strength over time in elite jumpers in comparison to controls irrespective of the analyzed leg (i.e., greater residuals). Furthermore, the take-off leg showed statistically significant lower uniformity (lower CS) in adaptive changes of muscle strength in comparison to the control group although statistical significance was not achieved for the inter-group comparison of fluctuations in tendon stiffness ($P = 0.074$) and maximal tendon strain ($P = 0.092$). There is therefore evidence that the notable fluctuations of muscle strength in the athlete group were only partly matched by an adaptive response in the tendon.

A more detailed examination of our data, moreover, revealed that some elite athletes clearly showed higher fluctuations over time in maximal tendon strain especially in their take-off leg than other athletes and controls (see interquartile range and maximal value in **Figure 2**). Accordingly we decided in addition to use an approach based on median split for CS for the take-off leg of the elite athletes and divided them into two groups: Low CS ($n = 8$) and High CS ($n = 9$) athletes (see **Figure 7**). Using this approach we found statistically significant greater fluctuations of muscle strength ($P = 0.007$) and tendon stiffness ($P = 0.032$) for Low CS elite jumpers in comparison to controls (**Figure 7**). As a consequence, tendon strain level during maximal contractions recorded over time showed in athletes significantly greater fluctuations as well ($P < 0.001$; **Figure 7**); there were episodes for which levels of strain were up to 1.5 times higher in comparison to controls. Given that tendon strain is accepted as the main mechanical parameter determining tendon damage accumulation (Wren et al., 2003; Andarawis-Puri et al., 2012), this implies to a temporarily increased mechanical demand on the tendon over a season and hence a greater threat to the tissue integrity. Moreover, the findings from our additional two elite athletes with chronic AT tendinopathy confirmed previous reports of a decreased tendon stiffness and increased tendon strain in patients with tendinopathy (Wang et al., 2012; Kulig et al., 2016). The above findings, when combined with the relatively high prevalence (up to 40%) of AT overuse in elite sprinters and jumpers (Janssen et al., 2018) support the notion that unbalanced adaptation of muscle and tendon in elite jumpers may have a clinical relevance and increase the risk for overuse injuries.

Given that fluctuation of muscle strength was more predominant than in tendon stiffness, we suggest that the fluctuation of strain was primarily due to the variations in muscle strength rather than tendon stiffness. Taking into account that tendons have a lower tissue turnover than muscles (Heinemeier et al., 2013) we argue that muscular adaptation takes place at a higher rate in response to modified mechanical loading induced by variations in training volume and content over a season. Regarding this, it is, however, important to note that the six elite athletes that we monitored over 4 years showed no indications for different temporal dynamics in muscle and tendon adaptation between the preparation and competition periods (**Figure 4**). These athletes demonstrated no significant differences in fluctuations of muscle strength and tendon stiffness and hence maximal tendon strain between preparation and competition periods, irrespective of the leg analyzed. Therefore, while we argue that the above observed non-uniform adaptation in elite athletes could be defined by the differences in the MTU temporal dynamics, potential variations in the training stimulus between preparation and competition were insufficient to cause measurable differences in the adaptive changes within the MTU in elite jumpers.

Adaptation of the MTU to various mechanical alternations has been the subject of numerous studies (Bohm et al., 2015a) and has provided evidence that the muscle and tendon homeostatic

response to mechanical loading can vary considerably. For example, a discrepancy between muscle and tendon in the time course of adaptation in response to mechanical loading has been reported previously for both the patellar and the Achilles tendon (Kubo et al., 2012), revealing that muscle strength gains can precede significant changes in tendon biomechanical properties. While an increase in tendon stiffness relies on modulation of tissue metabolism and subsequent adaptive changes of tendon structure and morphology (Kjær et al., 2009), neuronal adaptation enables marked increases in muscle strength even before major morphological changes occur within a muscle (Moritani and DeVries, 1979; Narici et al., 1989). Moreover, there is strong evidence that muscles tend to have superior plasticity in response to a wide range of exercise modalities in comparison to tendons, as both high and low loading magnitudes can facilitate increases in muscle strength but only high magnitude loading promotes tendon adaptation (Arampatzis et al., 2007a). Therefore, in line with recent studies conducted on the patellar tendons of adolescent volleyball players (Mersmann et al., 2016, 2017), we propose that injury prevention strategies in elite track and field athletes might need to integrate mechanical stimuli which primarily focuses on effectively increasing the tolerance of the tendon to mechanical loading i.e., by incorporating exercise modalities involving optimal strain magnitudes over longer strain durations (Arampatzis et al., 2007a; Bohm et al., 2014).

Interestingly, the individual tendon strain in the take-off leg showed greater fluctuations over 1 year of athletic training and competition for most but not for all elite athletes in comparison to controls (**Figure 7**). Moreover, the differences to control subjects were in absolute terms obvious for the swing leg but did not reach statistical significance (**Figure 2**). This substantial variation amongst elite athletes indicates that the relative time courses of adaptation in muscle and tendon might reveal differences at an individual level and that imbalances between muscle and tendon could often remain undetected when only group mean values are considered. Therefore we argue that individualized approaches (Arampatzis et al., 2020) and rapid subject-specific tendon strain estimates (Pizzolato et al., 2020) are needed to provide valuable information for coaches and athletes as well as their medical teams about the adaptive processes in muscles and tendons during the various phases of athletic training. This would enable detection of temporal disruption of the fine-tuned interaction within the MTU during the course of training and could allow adjustment of individual athlete training loads through tailored intervention.

With regard to our case study reporting of two elite athletes with unilateral chronic AT tendinopathy, we saw no evidence for differences in contractile strength between limbs but there was lower tendon stiffness in the affected limb. As a consequence, increased tendon strain during maximal contraction was identified for the affected limb, in accordance with previous results for both non-active adults and for athletes with tendinopathy (Wang et al., 2012; Kulig et al., 2016). Moreover, we were able to expand those findings, showing that AT tendinopathy is accompanied by lower uniformity (lower CS) in the adaptation changes of muscle strength and tendon stiffness, leading to higher fluctuations in tendon strain over time. This further supports the notion that an unbalanced adaptation within the MTU in athletes could heighten the risk for overuse injuries. In contrast to this, the case report data of one elite jumping athlete 10 months after AT rupture and reconstruction indicated opposing findings: there was a lower force generation capacity but a higher tendon stiffness in the affected limb, leading to a lower level of strain. These bilateral differences and deficient contractile strength in the affected limb seemed persistent as the athlete did not show any adaptive improvements over a follow up period of 2.5 years post-surgery (**Figure 6**). Similar findings have been obtained in several cross-sectional and longitudinal investigations of non-active AT rupture patients even more than 10 years post rupture and reconstruction (Agres et al., 2015; Heikkinen et al., 2017). As the current longitudinal data were generated from an elite athlete encountering athletics training with high training loads and volumes over a period of 2.5 years post-surgery we may argue that AT rupture and reconstruction lead to irreversible MTU dysfunction. In contrast to an injury or pathology, an unbalanced adaptation of muscle and tendon due to athletic training in elite jumpers seems to be a temporary phenomenon. Further long-term investigations are needed to determine whether targeting balanced development of muscle strength and tendon stiffness through individualized MTU-focused exercise interventions can enhance the effectiveness of tissue recovery caused by injury or prevent overuse tendon injuries in athletes.

In relation to our testing protocol one could argue that the used 90° knee joint configuration could place the m. gastrocnemius medialis to an unfavorable position to generate force based on its force-length relationship, which might lead to a difference in tendon subcomponent deformations and affect the determined tendon stiffness. In justification, the primary reason for using the flexed knee joint angle configuration was to minimize the inevitable ankle joint rotation that occurs when using an extended knee joint, as this causes a substantial overestimation of the tendon elongation due to the generated force during the plantar flexion contractions. In an earlier study (Ackermans et al., 2016) we investigated the current (90°) and a more dorsiflexed ankle joint configuration (85°) with the same knee joint setup to enhance the gastrocnemii force potential and their contribution to the resultant joint moment. Even though the more dorsiflexed position demonstrated significantly higher ankle joint moments and tendon elongation, no significant differences were found for tendon stiffness between those two joint configurations (Ackermans et al., 2016; **Supplementary Material** of the current study). Hence, although a flexed knee joint could potentially influence the interactions between the individual AT subtendons, it seems not to cause a clear measurable alteration in the m. gastrocnemius medialis tendon force-elongation relationship during loading, particularly in the "linear" region were the tendon stiffness was determined. However, we cannot exclude that the observed higher tendon strain fluctuations in elite athletes may be influenced also by variations in the regional strain patterns and subtendon sliding (Handsfield et al., 2020; Maas et al., 2020). Incorporating regional and subtendon strain differentiation could contribute additional understanding into the complex tendon loading pattern during

various contractions in elite athletes. Further, the current work made use of generic AT moment arms from previous literature (Maganaris et al., 1998), which might affect the calculated tendon stiffness in absolute terms. However, we believe this could not affect the main outcomes from our longitudinal analyses and the non-uniformity of muscle and tendon adaptations in mature elite athletes. Finally, it is important to note that only six elite athletes were considered in our comparison between preparation and competition periods, irrespective of our monitoring both limbs over a period of 4 years of athletic training. Further studies are needed to investigate whether potential variations of training volume and content between preparation and competition can lead to amplification of non-uniformity in adaptation of muscle and tendon. This may be of particular relevance as it has been reported that the frequency of musculoskeletal injuries occurring during preparation is threefold greater in comparison to competition in elite track and field athletes (D'Souza, 1994).

CONCLUSIONS

In conclusion, the current findings demonstrate that repetitive plyometric and habitual mechanical loading during training and competition can affect uniformity within the MTU in elite jumpers, leading to a temporarily increased demand on the tendon and potentially higher injury risk. We argue that the mechanism for unbalanced MTU adaptation during athletic training is related to differences in the time courses of muscle and tendon adaptation, which needs to be considered in exercise and rehabilitation. Further long-term investigations should address the potential of more individualized exercise interventions for prevention of disruptions to the fine-tuned interactions within the MTU in athletes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by German Sport University Cologne Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KK and GE conception and design of research, performed experiments, analyzed data, interpreted results of experiments, prepared figures, drafted manuscript and approved final version of manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.607544/full#supplementary-material

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Conflict of Interest: KK has equity in Protendon GmbH & Co. KG, whose measurement device and software was used for the data processing and analysis in this study.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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