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Abstract

The mechanical output of the muscle-tendon unit (MTU) is in part governed by the viscoelastic nature of tendinous tissues. The tendon contributes to beneficial contractile conditions during various types of movement. Yet, knowledge about the modulation of muscle-tendon behaviour under different external constraints and with different tendon stiffness is scarce. Moreover, the role of human tendons has mainly been examined in the context of energy conservation or power amplification and has not been investigated during tasks requiring energy dissipation. Thus, the aim of the present thesis was to examine muscle-tendon interaction: during a landing task; under external constraints (i.e. loading and speed) during landing and running; and after a training-induced increase in tendon stiffness during landing and running.

We conducted a cross-sectional study (A) and a controlled longitudinal study (B), to investigate muscle-tendon interaction during a landing task (n=39 and n=21, respectively) and during running on a treadmill (n=16 and n=21, respectively). In study A, ankle and knee joint mechanics and gastrocnemius and soleus MTU length were derived from kinematic and kinetic data during both tasks. In addition, we synchronised ultrasound with these data to measure Achilles tendon and muscle fascicle length. Loading (+20% of body mass) was added to the subjects using weighted vests after completing the tasks unloaded. Running was performed at preferred speed and increased speed (+20%). In study B, the training group (n = 11) underwent 10 weeks of resistance training consisting of single-leg isometric plantar flexion contractions. Similar measurements as in study A were performed during unloaded landing and unloaded running at preferred speed, additionally including the assessment of plantarflexion force, tendon stiffness and strain, measured during isometric contractions. Repeated-measures ANOVAs were used to test differences in the variables of interest between conditions of task execution (study A) and between baseline and post-training tests (study B).

During landing in study A, the rapid MTU stretch was taken up by the Achilles tendon, while gastrocnemius and soleus fascicles lengthened actively at slower velocities than the MTU. Loading resulted in a greater tendon stretch and higher soleus muscle activity to compensate for the greater negative work during landing, whereas fascicle strains remained unchanged. During running, loading resulted in an increased Achilles tendon recoil – but not elongation -, whereas higher running speed resulted in greater gastrocnemius and soleus muscle activity. Fascicle length and velocity of either muscles were unchanged when imposing external constraints during running. In study B, Achilles tendon stiffness increased by 18%, without a significant reduction in tendon strain during isometric contractions. Increased resting pennation angle and muscle thickness (by 5% each) were accompanied by a 15% increase in plantarflexion strength in the training group but fascicle length was not altered by the training. During landing, longitudinal tendon strain remained similar after training, despite unchanged tendon force and increased tendon stiffness. Fascicle lengthening and velocity were reduced (by 27 and 21%, respectively) after training and, surprisingly, gastrocnemius fascicle length at touchdown was greater (8%). No differences were observed for soleus. During running, tendon elongation did not change after training but tendon recoil was reduced by 30%. Estimated tendon force was similar after training and neither gastrocnemius nor soleus fascicle shortening were affected. Altered gastrocnemius muscle mechanics were nevertheless indicated by greater changes in pennation angle and a higher architectural gear ratio measured during stance. None of the variables changed in the control group after the training duration.

The results showed that during landing, the rapid MTU stretch was buffered by the Achilles tendon to delimit muscle fascicle strains. Additional loading was taken up by the tendon and was met by increased soleus muscle activity, effectively preserving the contractile length and velocity of muscle fascicles. Similarly, fascicle behaviour was preserved during running with load and at increased speed. We observed different strategies to increase ankle joint work under the two constraints; higher elastic energy utilisation was favoured with added load, whereas increasing speed resulted in higher muscle activity. The training-induced increase in Achilles tendon stiffness also altered muscle-tendon behaviour during landing and running. During landing, the buffering action of the tendon was not limited despite similar forces and increased tendon stiffness. Mechanisms for the reduction in gastrocnemius lengthening and greater length at touch-down during landing remain to be determined. During running, gastrocnemius and soleus fascicle shortening patterns were preserved, despite a reduced tendon recoil. Increased gastrocnemius gear ratio during stance may explain asymmetrical changes in tendon stretch and recoil and may suggest that elastic energy was stored differently after training.

Within the limits of the experimental conditions of the present thesis, fascicle behaviour seems little affected by changes in external constraints (i.e. speed and load) or increased tendon stiffness during landing and running, whereas soleus seems less affected than gastrocnemius. Instead, most conditions were modulated by elastic energy storage or by muscle activity. We suggest that energy storage and contraction behaviour were influenced by changes in aponeurosis stiffness and can therefore not be fully explained by simple in series models.

Sammendrag (abstract in Norwegian)

Den mekaniske funksjonen til muskel-sene enheten er knyttet til senevevets viskoelastiske egenskaper. Senen bidrar til å forbedre arbeidsforholdene for musklene i ulike typer bevegelse. Likevel er kunnskapsgrunnlaget som undersøker endringer i muskel-sene adferd ved ulike eksterne begrensninger og ved ulik senestivhet mangelfullt. I tillegg har rollen til menneskets sener hovedsakelig blitt undersøkt i sammenheng med energilagring eller kraftforsterking, ikke under oppgaver som krever energidissipasjon. På bakgrunn av dette var målet med denne avhandlingen å undersøke muskel-sene interaksjon: under en landingsbevegelse; under eksterne begrensninger, som ekstra vekt og økt fart, i landing og løping; og med økt senestivhet i landing og løping.

Vi gjennomførte to studier, en med tverrsnitts design (A) og en med kontrollert longitudinelt design (B), for å undersøke muskel-sene adferd ved en landingsbevegelse (n=39 og n=21) og ved løping (n=16 og n=21). I studie A ble ankel- og kneledd-mekanikk og lengden til muskel-sene-enhet til gastrocnemius og soleus målt ved bruk av kinematiske og kinetiske målemetoder, under begge øvelser. I tillegg synkroniserte vi målingene med ultralyd for å måle lengden til Akillessenen og muskel-fasiklene. Vi la til ekstra vekt (+20% av kroppsvekt) på forsøkspersonene etter at de gjennomførte begge øvelsene uten ekstra vekt. Forsøkspersonene ble først bedt om å løpe med foretrukket fart, og deretter ble farten økt med 20%. I studie B utførte treningsgruppen (n = 11) et 10-ukers treningsprogram som bestod av ett-bens isometriske plantar-fleksjons kontraksjoner. En lignende testprotokoll som i studie A ble brukt, som i tillegg inkluderte målinger av kraft ved plantarfleksjon og senestivhet. Repeterte-målinger ANOVAs ble brukt til å teste forskjeller i utvalgte variabler mellom de ulike begrensningene satt for utførelse av oppgaven og mellom testene pre- og post intervensjon.

Under landingsøvelsen i studie A ble den raske forlengningen av muskel-sene-enheten tatt opp av Akillessenen og gastrocnemius og soleus ble aktivt forlenget med langsommere hastigheter enn muskel-sene-enheten. Ekstra vekt resulterte i en større forlengelse av senen og høyere muskelaktivitet i soleus for å kompensere for det økte negative arbeidet i landingen, mens lengdeendringen i muskelfasikler var lik. Under løping førte ekstra vekt til økt recoil i Akillessenen men ikke økt lengdeendring. Derimot resulterte høyere løpshastighet i høyere muskelaktivitet i både gastrocnemius og soleus. Fasikkellengden og fasikkelhastighen i begge musklene var uforandret med økt belastning eller økt fart under løping. I studie B økte stivheten

i Akillessenen med 18%, uten en signifikant reduksjon i senens lengdeendring etter treningen. En økning i pennasjonsvinkelen og muskeltykkelsen i hvile (5% hver) var ledsaget av en 15% økning i styrke i plantarfleksorene for treningsgruppen, men fasikkellengden forble uforandret etter treningsintervensjonen. Fasikkel-forlengelse og fasikkelhastighet ble redusert (med 27 og 21%) og gastrocnemius fasikkellengde var lengre ved begynnelsen av landingen (8%) etter treningsintervensjonen. Vi fant ingen forskjeller for soleus muskelen. Under løping var senelengden uforandret etter treningen, men recoil i senen var redusert med 30%. Den estimerte senekraften var lik etter treningsintervensjonen og forkortning av fasikler i både gastrocnemius og soleus ble heller ikke påvirket av treningen. En endring i muskelmekanikken i gastrocnemius ble likevel indikert ved en større endring i pennasjonsvinkel og en høyere arkitektonisk gear ratio under bakkekontaktfasen. Ingen variabler var endret i kontrollgruppen.

Resultatene viste at den raske forlengningen av muskel-sene-enheten i landingen ble bufret av Achilles-senen for å redusere høye strekkhastigheter i muskelfasiklene. Ekstra vekt ble tatt opp av senen og ble samtidig møtt av økt muskelaktivitet i soleus slik at lengden og fasikkelhastigheten kunne bli opprettholdt. Fasikkeladferden var også upåvirket under løping med ekstra vekt og med økt hastighet. Vi observerte ulike strategier for å øke arbeid i ankelleddet under de to begrensningene; når ekstra vekt ble påført, ble høyere elastisk energiutnyttelse favorisert, mens økt fart resulterte i høyere muskelaktivitet. Den treningsinduserte økningen i Akillessenestivhet endret også muskel-seneadferd under landing og løping. Under landingsøvelsen ble bufferfunksjonen til senen ikke redusert, på tross av lignende senekrefter og økt senestivhet. Vi kan på bakgrunn av denne studien ikke si noe om mekanismene som ligger til grunn for reduksjonen av gastrocnemius forlengelse og økt lengde ved begynnelsen av landingen. Under løping ble forkortningsmønstre i gastrocnemius og soleus fasikler bevart, til tross for den reduserte seneforlengningen. Økt gastrocnemius arkitektonisk gear ratio under bakkekontakt kan forklare de asymmetriske endringene i seneadferd og kan tyde på at måten elastisk energi ble lagret på var endret etter treningen.

Innenfor grensen av det eksperimentelle oppsettet i denne oppgaven virker musklenes fasikkeladferden til å bli lite påvirket av ytre begrensninger (dvs. økt fart og ekstra vekt) eller økt sene stivhet under landinger og løping, mens soleus virker mindre påvirket enn gastrocnemius. I stedet var de fleste betingelsene (økt fart, ekstra vekt og økt senestivhet) modulert av elastisk energilagring eller av muskel aktivitet Vi foreslår derfor at energilagring og kontraksjonsadferd ble påvirket av endringer i aponeurose stivhet og kan derfor ikke fullstendig forklares med enkle modeller som har muskel og sene i serie.

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Oslo, September 2018

Amelie

List of articles

Article 1

Werkhausen, A., Albracht, K., Cronin, N. J., Meier, R., Bojsen-Møller, J., & Seynnes, O. R. (2017). Modulation of muscle-tendon interaction in the human triceps surae during an energy dissipation task. *The Journal of Experimental Biology*, 220(22), 4141.

Article 2

Werkhausen, A., Albracht, K., Cronin, N. J., Paulsen, G., Bojsen-Møller, J., & Seynnes, O. R. (2018). Effect of Training-Induced Changes in Achilles Tendon Stiffness on Muscle–Tendon Behavior During Landing. *Frontiers in Physiology, 9,* 794.

Article 3

Werkhausen, A., Cronin, N. J., Albracht, K., Bojsen-Møller, J., & Seynnes, O. R.. Distinct muscle-tendon interaction during running at different speeds and in different loading conditions. In review *Journal of Applied Physiology*.

Article 4

Werkhausen, A., Cronin, N. J., Albracht, K., Paulsen, G., Larsen, A.V., Bojsen-Møller, J., & Seynnes, O. R.. Training-induced increase in Achilles tendon stiffness affects tendon strain and muscle contraction behaviour during running. Submitted to *PeerJ*.

Glossary

	Abbreviation
Achilles tendon	**AT**
Analysis of variance	**ANOVA**
Architectural gear ratio during stance	**AGRs**
Coefficient of variation	**CV**
Elastic elements	**EE**
Electromyography	**EMG**
Fascicle	**f**
Gastrocnemius medialis	**GM**
Ground reaction force	**GRF**
Maximal voluntary contraction	**MVC**
Muscle-tendon unit	**MTU**
Soleus	**SOL**
Standard deviation	**s.d.**
Standard error of the mean	**s.e.m.**
Tibialis anterior	**TA**

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1 Introduction

Over recent decades, our understanding of in vivo tendon behaviour increased by virtue of significant improvements in technology to explore muscle and tendon tissue properties and behaviour. Specifically, it has become clear that tendinous tissue not only acts as a force transmitter. Muscle-tendon interaction during tasks such as jumping (Aerts, 1998; Astley & Roberts, 2012), running (Roberts et al., 1997) or landing (Konow et al., 2012) has first been investigated in animals. Depending on the requirements of the movement, tendinous tissues can fulfil various roles that Roberts and Azizi (2011) have categorised based on energy flow as movements requiring power amplification, energy conservation or power attenuation. The flexible mechanisms show that tendon not only enables the storage of elastic energy, it uncouples muscle length changes from that of the muscle-tendon unit (MTU) to enhance muscle performance. Consequently, changes in external constraints or tendon mechanical properties potentially affect elastic energy storage and muscle contractile behaviour during various tasks as indicated by observations during walking and running on different slopes (Lichtwark & Wilson, 2006) and by *in silico* data that shows the effects of varying series elasticity (Lichtwark & Wilson, 2008). More information about the role of tendon stiffness is required, especially when considering the possible constraints under which different tasks can be performed and the plasticity of tendon, which results from loading (Wiesinger et al., 2015).

1.1 Background

This section aims to present the current knowledge about muscle and tendon properties and the diverse interaction mechanisms of the structures during locomotion to identify why the modulation of muscle tendon interaction is important. Muscle force production capacity, tendon properties and plasticity of muscle and tendon will be described separately before linking their function. The tissue properties discussed here, primarily apply to pennated muscles with relatively long and elastic tendons such as the triceps surae MTU, which represents an essential component of human locomotion and was examined in this thesis.

1.1.1 Contractile and elastic properties of the MTU

The structure of human muscle and tendon has been studied for centuries mainly by the means of anatomical dissections by anatomists and physiologists. The development and improvement of technology has made *in vivo* investigation of muscle and tendon behaviour possible.

Especially the use of imaging techniques has rapidly increased during the last decades and allowed us to gain a better understanding of MTU function. Muscle, more recently tendon, and lately aponeurosis have been examined using different methods that are constantly improving.

1.1.1.1 Muscle contractile properties and plasticity

Sarcomeres are the smallest functional unit of the muscle and its structure is crucial for function. The striated structure consists of repeating arrays of thin actin and thick myosin filaments which are partly overlapping and connected through cross-bridges shaping myofilaments (Huxley, 1957). In combination with other proteins, these myofilaments form a myofibril. The attachment of myosin heads to the actin chain and the relative rotation of the filaments results in sarcomere shortening and generates active tension. The process has been discovered by two independent research groups and is known as the sliding filament theory (Huxley & Niedergerke, 1954; Huxley, 1953; Huxley, 1969). More recently, the distinct role of the titin filament has received increasing attention and its importance for muscle force regulation is not yet completely understood. Herzog et al. (2012) demonstrated the important role of titin during eccentric contractions and at long muscle lengths. A primary role of titin is passive force production, whereas its contribution to residual force enhancement still remains controversial (Herzog, 2018). The particular position of filaments, such as the overlap in different joint configurations, has functional consequences for the force production capacity of the muscle.

Due to variation in the overlap of actin and myosin filaments during contractions, muscle force production is length dependent, which has been described as the force-length relationship of muscles (Gordon et al., 1966). The human gastrocnemius and soleus muscle e.g. have been shown to operate at the ascending limb of the force-length relationship (Arnold et al., 2013; Herzog et al., 1991; Maganaris, 2003a; Rubenson et al., 2012), although limitations for estimating the joint torque (i.e. determination and changes of moment arm during contraction and force contribution from other muscles) make accurate measurements difficult.

The force producing capacity of muscles is not only length dependent: Hill could already in 1938 demonstrate the dependency on contraction velocity (Hill, 1938). This relationship of hyperbolic shape shows how muscle actions are constrained to force and velocity output. The maximum force a muscle can generate, decreases with increasing shortening velocity, whereas the contraction velocity decreases with increasing force (Hill, 1938). Power output of a muscle is therefore maximised at approximately 30% of its maximum shortening velocity (Hill, 1964). Higher force than that obtained during isometric conditions have been attained for eccentric muscle actions, although results may depend on whether a constant velocity or force were required from the muscle (Edman et al., 1978). Higher force production during eccentric contractions has partly been explained by residual force enhancement, a history dependence property of muscles. Contrarily, residual force depression was found for concentric contractions. However, the hypothesis of history dependent muscle behaviour cannot be fully explained with current cross-bridge theory for muscle contractions. While several mechanisms have been proposed (Herzog, 1998), the structural protein titin is suggested to play an important role in history dependent muscle behaviour (Herzog et al., 2016).

Both force-length and force-velocity relationship depend on muscle architecture i.e. the spatial organization of muscle fibres and their orientation in relation to the axis of muscle force generation. Hence, structural arrangement fundamentally determines the function of a muscle (Burkholder et al., 1994; Lieber & Brown, 1992). The number of sarcomeres in parallel is proportional to the maximum force a muscle can develop because each parallel sarcomere contributes to force production. Conversely, the number of sarcomeres in series is proportional to the muscles maximum shortening velocity and excursion range because the excursion of the in-series sarcomere determines excursion of the whole muscle.

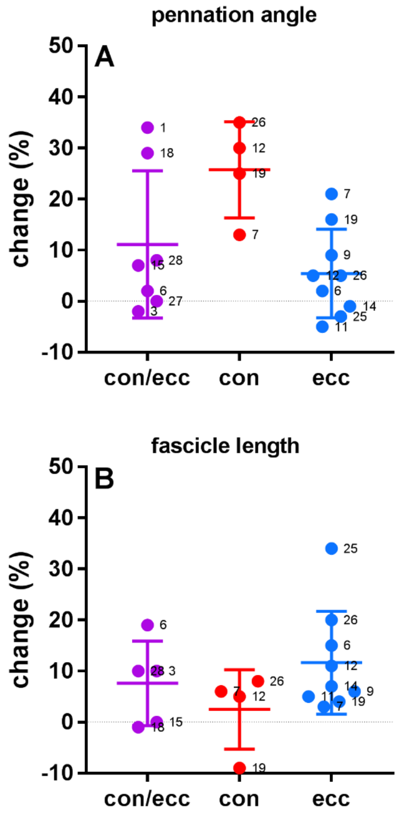
To provide estimates of force production and excursion capacity of a muscle based on the relationship of sarcomeres in parallel and in series, scientists have determined fascicles length by imaging the muscle. Fascicle length is determined along its axis between the superficial and deep aponeurosis. Although sarcomere length is variable within a muscle and the number of sarcomeres per fibre is region dependent, fascicle length changes are moderately correlated to sarcomere length changes (Lichtwark et al., 2018). Thus, fascicle length may provide a valuable estimate of number of sarcomeres in series. The longitudinal curvature of fascicles, which tends to straighten when the muscle is activated, may explain the small regional differences. This irregular fascicle curvature is suggested to depend on the resistance sites along the fibre (Gans, 1982). Fascicle curvature is often eluded for methodological convenience when fascicle length is examined (Rana et al., 2014).

The orientation of the fascicle relative to the line of action of the MTU is defined as the pennation angle, which also defines a muscles architecture. The pennation angle of a muscle is usually defined as the angle between the fascicle and the aponeurosis that is more closely aligned with the direction of the force produced by bipennate muscles. The common two-dimensional definition of pennation angle provides a crude estimation of the fibre orientation in the three-dimensional muscle. Pennation allows for more muscle fibres along the longitudinal axis of a muscle (Lieber & Fridén, 2000), which increases the physiological cross-sectional area (assuming the same muscle volume) and thereby the maximum force capacity (Ikai & Fukunaga, 1968; Wickiewicz et al., 1983). Yet, a drawback of the pennate design compared to a parallel fascicle arrangement is that fascicle force is not directed in the muscle's line of force but reduced by the cosine of the pennation angle. However, the packing of the contractile proteins compensates for this until a pennation angle of up to 45°. Hence, the structural arrangement of contractile elements has important implications for muscle force production. *In vivo* measurements have shown large variations between subjects for the structural arrangement of sarcomeres as both fascicle length and pennation angle differed (Narici et al., 1996).

During contractions the spatial arrangement of fascicles changes, e.g. fascicles shorten during a concentric contraction. To maintain a constant muscle volume, muscle fascicles have to expand transversally when they shorten (Huxley, 1953). Therefore, muscle shortening is not only due to fascicle shortening but often associated with fascicle rotation or rather a change in pennation angle. This mechanism resembles a functional muscle gearing (architectural gear ratio - AGR) and is most commonly quantified as the quotient of muscle velocity and fascicle velocity (v_{muscle} / $v_{fascicle}$) (Azizi et al., 2008). The rotation of muscle fascicles enables greater shortening velocities of the muscle than would be reached due to fascicle shortening only (AGR >1: muscle shortening velocity is higher than fascicle shortening velocity). Thus, a possible advantage of gearing is that constraints on sarcomere level on force and velocity production of the muscle can be circumvented. Gearing has furthermore been shown to be variable depending on the force requirements. When high force production is required, the muscle shifts to a low gear because the drawback of high gearing is that fascicle rotation reduces the force that is transmitted to the tendon when pennation angle increases (Azizi et al., 2008). Transverse (i.e. off-axis) forces have been suggested to determine variable gearing through variable shape changes in width and thickness of the muscle. The radial expansion of muscle fascicles is suggested to be modulated by intramuscular springs, which contributes to changes in thickness and width depending on the contractile load (Eng et al., 2018). In humans, the importance of three dimensional shape changes for gearing was first demonstrated during fixed-end contractions (Randhawa et al., 2013). Later, the force dependency of the dynamic muscle shape changes was established during cycling, additionally noting that velocity did not alter fascicle shape changes (Dick & Wakeling, 2017).

There is a consensus in the literature that muscle morphology and architecture change in response to chronical loading. These parameters have often been investigated in intervention studies using different resistance training protocols and connecting the changes to the force producing capacity. An increase in the muscle anatomical cross-sectional area in response to resistance training has been recognised decades ago (Narici et al., 1989; for the quadriceps) and seems to occur irrespective of contraction type of the training regimen (Wernbom et al., 2007). In pennate muscles, examining the physiological cross-sectional area may provide the best estimate of number of sarcomeres in parallel, due to its relation to the muscles force producing capacity. This relationship was confirmed by subsequent studies reporting an increased physiological cross-sectional area and increased muscle force after resistance training (e.g. Kawakami et al., 1995 for elbow extensors; Seynnes et al., 2009 for quadriceps muscles). Similarly, increased muscle thickness after resistance training seems to be connected to increased muscle force (e.g. Alegre et al., 2006 for vastus lateralis; Duclay et al., 2009 for gastrocnemius medailis).

Besides changes in muscle dimensions, pennation angle and fascicle length are important architectural parameters that have been shown to adapt to increased load (Aagaard et al., 2001; Blazevich & Giorgi, 2001). Most intervention studies report an increase in pennation angle following various resistance training protocols for different muscles. There is a lack of consensus between studies whether adaptations in pennation angle may depend on the contraction type (for review see Timmins et al., 2016). However, there may be a trend that concentric training increases pennation angle more than eccentric training (**Figure 1A**). The fact that similar training of vastus lateralis and vastus medialis induced changes only in the former muscle (Blazevich et al., 2007), further indicates a muscle-dependency for adaptations of pennation angle. This study showed a similar dependency on the muscle for fascicle length changes. Pennation angle change provides an indication about the number of sarcomeres in parallel and hence the capacity of a muscle to produce high forces, whereas fascicle length changes are associated with an increase in the number of sarcomeres in series and thus the muscles shortening velocity (Goldspink, 1985). Studies with different contraction modes suggest that eccentric training increases fascicle length, while the effect of concentric training on fascicle length was not consistent among studies (**Figure 1B**). Indeed, the lack of consistency between studies measuring the effect of training on muscle architecture may be ascribed to differences in training and testing modalities. Alternatively, the lack of consensus may suggest that other variables, which vary between interventions (e.g. range of motion and contraction velocity), should be considered (Sharifnezhad et al., 2014; Valamatos et al., 2018). Additionally, it is suggested that adaptations may be regional within a muscle (Blazevich et al., 2006), further impeding comparability between studies. Although several questions remain open about training modalities, **Figure 1** clearly shows that pennation angle and fascicle length are affected by resistance training demonstrating the potential of resistance training to modulate muscle contractile conditions.



(1)	Aagaard et al. (2001), vastus lateralis
(3)	Alegre et al. (2006), vastus lateralis
(6)	Baroni et al. (2013), vastus lateralis, rectus femoris
(7)	Blazevich et al. (2007), quadriceps femoris
(9)	Duclay et al. (2009), gastrocnemius medialis
(11)	Fouré et al. (2013), triceps surae
(12)	Franchi et al. (2014), vastus lateralis
(14)	Guex et al. (2016), biceps femoris long head
(15)	Guilhem et al. (2013), vastus lateralis
(18)	Kawakami et al. (1995), triceps brachii
(19)	Kim et al. (2015), supraspinatus
(25)	Potier et al. (2009), biceps femoris long head
(26)	Reeves et al. (2009), vastus lateralis
(27)	Rutherford and Jones (1992), quadriceps femoris
(28)	Seynnes et al. (2007), quadriceps femoris

Figure 1. *Effect of training interventions with different contractions modes (con - concentric, ecc – eccentric) on pennation angle (A) and fascicle length (B).*

1.1.1.2 Tendon properties and plasticity

Although tendon has historically been considered as inert tissue, research of the last decades has shown that the viscoelastic properties of force bearing tissues is decisive for storage and release of elastic energy and determining for the force generating capacity of muscles (Hof et al., 1983; Roberts et al., 1997). Tendon behaviour is characterised by composition and structure, quantified by the tendon dimensions (i.e. cross-sectional area and length) and the material properties (i.e. Young's Modulus) (Butler et al., 1978). The importance of tendon has especially been recognised for the lower limbs, where tendons are relatively long and compliant. Tendon tissue is composed of collagen (mostly type I) and elastin embedded in a proteoglycan-water matrix (Gelse et al., 2003; Tipton et al., 1975). The structure is organised in a complex hierarchy, where cross-links between collagen molecules aggregate into micro fibrils, which form collagen fibrils and then fibres (Hess et al., 1989).

Tendon dimensions and material properties determine the mechanical properties of tendon, which are a critical parameter for function. The most frequently reported parameter is tendon stiffness (as the inverse of compliance). The force-elongation relationship of superficial tendons such as the Achilles tendon can be assessed *in vivo* combining ultrasound and dynamometry. Typically, the relationship is comprised by a toe-region at lower force levels and a rather linear region at higher force levels (Fukashiro et al., 1995; Maganaris & Paul, 1999). Stiffness is calculated as the slope of the linear part of the force-elongation relationship, most commonly at forces of about 50-90% of the maximum force.

Research of the last decades has clearly demonstrated that tendon adapts to mechanical loading. In general, tendon stiffness has been shown to increase following different training protocols, however, adaptations are not clearly favouring a specific training mode (**Figure 2**). It has been proposed that high strains are necessary to induce increases in Achilles tendon stiffness (Arampatzis et al., 2007), whereas Wiesinger et al. (2015) conclude in their systematic review that although most training interventions caused sizeable increases in tendon stiffness, results did not allow conclusions about dose-response and time-course relations of the effect of loading on tendon. However, increases in tendon stiffness after short-term training (i.e. weeks) were associated with changes in material properties while increased stiffness following long-term training (i.e. years) was related to tendon hypertrophy. The differences in training protocols and assessment methodology between studies impede further conclusions. It is for example suggested that tendon cross-sectional area adapts locally to increased loading and unloading (Couppé et al., 2013; Kongsgaard et al., 2007), requiring a detailed standardization of measurement sites. Several other limitations such as tendon force estimation methods, determining tendon slack length and techniques to determine tendon length changes have to be considered when measuring tendon stiffness (for review see Seynnes et al., 2015). Nevertheless, it has been shown that distinct loading patterns over years result in specific properties of patellar and Achilles tendon. Increased tendon cross-sectional area was reported as a result of high loading intensity or volume, maintaining the structural integrity of the tendon (Wiesinger et al., 2016). However, further studies are required to clarify the mechanisms for adaptations of tendon properties with different training modalities.

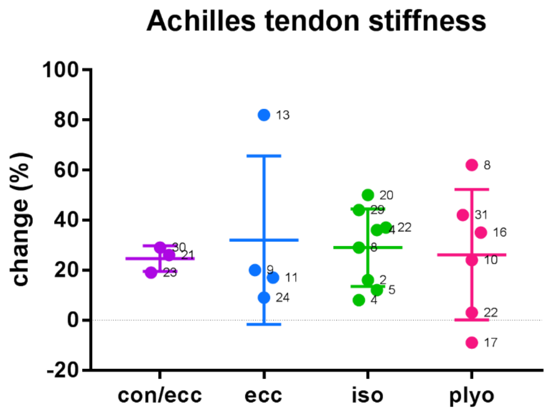


Figure 2. *Effect of training interventions with different contraction modes (iso - isometric, plyo – plyometric, con - concentric, ecc – eccentric) on Achilles tendon stiffness.*

(2)	Albracht and Arampatzis (2013)
(4)	Arampatzis et al. (2007)
(5)	Arampatzis et al. (2010)
(8)	Burgess et al. (2007)
(9)	Duclay et al. (2009)
(10)	Fouré et al. (2010)
(11)	Fouré et al. (2013)
(13)	Geremia et al. (2018)
(16)	Hirayama et al. (2017)
(17)	Houghton et al. (2013)
(20)	Kubo et al. (2012)
(21)	Kubo et al. (2010a)
(22)	Kubo et al. (2017)
(23)	Kubo et al. (2002)
(24)	Mahieu et al. (2008)
(29)	Waugh et al. (2017)
(30)	Waugh et al. (2014)
(31)	Wu et al. (2010)

Hysteresis is another important property of tendons. It describes the energy loss due to viscosity of the tissue. Despite the importance of hysteresis for the mechanical behaviour of the tendon, there is little literature available reporting *in vivo* hysteresis data from human tendons. A possible reason for limited knowledge may be methodological shortcomings (Finni et al., 2013). Although high standard deviations of these methods were reported, in vivo hysteresis measurements of human tendon have suggested a range of values with the mean between 12 and 19% (Farris et al., 2011 (17%); Kubo et al., 2014 (18-20%); Maganaris & Paul, 2000 (19%); Wiesinger et al., 2017 (12-18%)). However, hysteresis was first measured *in vitro* in various mammal tendons reporting lower values of 6-11% (Bennett et al., 1986; Ker, 1981; Pollock & Shadwick, 1994). Regardless, hysteresis seems to adapt to loading patterns. Specifically, increased loading may reduce hysteresis in human patella and Achilles tendon (Wiesinger et al., 2017).

1.1.1.3 The role of aponeurosis

The main connective tissue linking muscle fascicles to tendon is a continuous elastic sheet called aponeurosis. Forces exerted during a contraction are transferred via aponeurosis to the tendon, which suggests the relevance of this connective tissue in force transmission and function. Since fascicles affect aponeurosis loading during contractions and vice versa, the loading patterns are likely more complicated than that of the uniaxially loaded free tendon. When muscle fascicles rotate during contractions, the muscle bulges due to the isovolumetric constraint of muscles (Matsubara & Elliott, 1972), which affects aponeurosis shape as previously described. Yet, aponeurosis tissue behaviour and properties have received little attention in the literature (Raiteri, 2018). After differential strain patterns of the free tendon and the gastrocnemius aponeurosis were measured in humans (Magnusson et al., 2003), transverse aponeurosis strains were first demonstrated using *in vitro* fluoroscopy measurements of the turkey gastrocnemius (Azizi et al., 2009). The authors of this study suggest that the force dependency of aponeurosis stiffness modulates longitudinal stiffness of the aponeurosis and therefore affects muscle contractile conditions.

Different methodological approaches were used to examine the role of aponeurosis for MTU function. *In situ* experiments showed that at low and intermediate force levels, the increase in aponeurosis width was proportional to fascicle shortening (Arellano et al., 2016). Transverse aponeurosis strains at submaximal force levels were also reported in human in vivo studies (Farris et al., 2013; Maganaris et al., 2001). Furthermore, indirect evidence from an animal study (Ettema & Huijing, 1989) and recent data on the human tibialis anterior (Raiteri et al., 2018) suggest a connection between muscle-tendon unit length and aponeurosis stiffness. Another recent study could elegantly show a relationship between muscle shape changes and aponeurosis mechanical properties at high forces (Eng & Roberts, 2018). Hence, aponeurosis shape changes seem closely connected to dynamic shape changes that are related to muscle gearing (as described in chapter 1.1.1.1). Besides gearing, other factors such as spatial variability of force, differences in strain distribution, local muscle activation or external compressions possibly alter aponeurosis behaviour and function or vice versa (Raiteri, 2018).

Evidence from the above mentioned *in situ* and *in vivo* studies suggests that aponeurosis stiffness is variable in longitudinal and transverse direction and thus alters force transmission from muscle fascicles to the tendon. To date, the implications of variable aponeurosis stiffness for locomotion remain unknown. In this context, it seems likely that aponeurosis mechanical properties can be altered by long-term application of load, similar to muscle and tendon. This is supported by the finding of increased transverse aponeurosis stiffness during contractions of old compared to young rats (Holt et al., 2016) but remains to be examined in other contexts.

1.1.2 Muscle-tendon interaction during locomotion

The importance of linking muscle behaviour with connective tissue has first been recognised in animals (Alexander & Bennet-Clark, 1977; Alexander & Vernon, 1975), followed by the discovery of more diverse roles of elastic elements in muscle-tendon interaction (Roberts & Azizi, 2011). To describe the interaction of the different components of the MTU, typically a three-element muscle-tendon model is used (

Figure 3), here with the common example of the triceps surae. The contractile elements are the triceps surae muscles soleus and the gastrocnemii, which have relatively short pennated muscle fibres. The long and compliant Achilles tendon is the elastic element located in series with the muscle and other elastic tissue constitute the parallel elastic component. As mentioned earlier (1.1.1.3) the critical role of aponeurosis has not received much attention compared to the series elastic elements, therefore this section will mainly focus on the interaction between muscle and tendon.

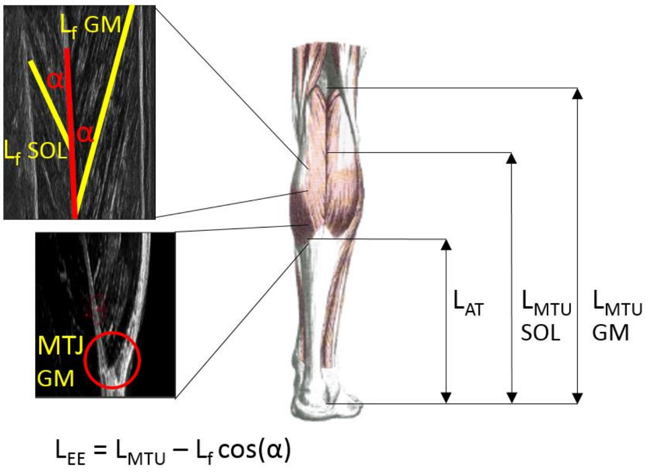


Figure 3. *Model of the triceps surae muscle-tendon unit (MTU) with soleus (SOL), gastrocnemius medialis (GM) and Achilles tendon (AT). Ultrasound images illustrate the myotendinous junction (MTJ) of the fascicles (f) of GM and SOL with their respective pennation angle (α) ((adapted from Lichtwark & Wilson, 2007). Length of elastic elements (EE) was originally calculated by Fukunaga et al. (2001).*

Beyond the function as force transmitter, tendon was long considered as ‘boring’ tissue, one dimensional in function and inert to mechanical constraints. Yet, when tendons are stretched, they develop a passive tension owing to their properties. Roberts and Azizi highlighted the role of tendon elegantly by writing: “What is so fascinating about elastic mechanisms is that, from such a simple and arguably limited mechanism, nature has produced a remarkable variety of applications” (Roberts & Azizi, 2011, p.353).

Muscles actively generate forces, but their power output is constrained to the earlier discussed force-velocity and force-length relationship. Combining the particular properties of tendon with that of the muscle allows the MTU to operate beyond the intrinsic muscle constraints by decoupling the timing of muscle work production and constraints from joint kinematics. Although maximum power production capacity of muscles is at about one-third of maximum shortening velocity, muscles often operate isometrically or at slower velocities when producing high forces, to maximise force output and economy of the whole MTU (Biewener et al., 1998; Roberts et al., 1997). This interaction has been illustrated by e.g. Farley and colleagues by comparing the function of tendon springs in the MTU with a person hopping on a pogo stick (Farley et al., 1991). The following chapters are structured according to the framework provided by Roberts and Azizi categorizing the diverse functions of elastic elements during locomotion based on the energy flow (Roberts & Azizi, 2011).

1.1.2.1 Energy conservation

Energetic and mechanical studies were the first to attribute cyclical work during running to elastic structures (Alexander & Vernon, 1975; Cavagna et al., 1964), before muscle fascicle behaviour was directly measured in animals during running (Roberts et al., 1997) and hopping (Biewener et al., 1998). During these movements, energy is temporarily stored in the tendon and then returned to the body. These findings were subsequently supported by measurements of the human triceps surae behaviour during walking (Cronin et al., 2013; Fukunaga et al., 2001), running (Lai et al., 2018; Lichtwark et al., 2007) and hopping (Lichtwark & Wilson, 2005b). In fact, these studies demonstrated that muscle fascicles of the triceps surae muscles shorten or act nearly isometrically during the ground contact phase. At the same time, elastic elements are first stretched and they subsequently recoil during the push-off phase as could be expected from joint kinematics. The Achilles tendon appears to account only partly for the elastic stretch and recoil, suggesting that other elastic elements, such as aponeurosis, significantly contribute to MTU elasticity (Lichtwark & Wilson, 2006). Distinct mechanistic functions of the muscles within the triceps surae MTU were indicated by faster fascicle shortening velocities of gastrocnemius compared to soleus during walking and (Cronin et al., 2013) running (Lai et al., 2018). Notably, the soleus MTU is stretched more than that of gastrocnemius, which may be attributable to the bi-articular nature of the latter muscle whose lengthening due to ankle dorsiflexion is offset by knee flexion after touch-down (Lai et al., 2018).

The obvious advantage of storage and release of elastic energy in the tendon is that work production is reduced for the muscle. Although work can be done by elastic tissues, muscles still have to generate force to provide tension for the tendon in series. However, when muscles perform less work, their energetic requirements are lower (Fenn, 1924). Additionally, at lower fascicle shortening velocity, the same force can be produced with less activation (Gabaldón et al., 2008) owing to the force-velocity relationship of muscles. Taken together, the system of contractile tissue in series with elastic force bearing tissues allows muscle fascicles to operate at more favourable velocities. Although fascicles shortened more during running compared to walking, the contraction velocity is still low relatively to maximum speed of the muscle fascicles (Lichtwark et al., 2007; Zajac, 1989). The decoupling of timing of muscular work and locomotor work by the temporary storage of elastic energy supports energy efficient development of power. Work input and output of the tendon are relatively similar (except of slight energy loss due to hysteresis) and so is the mechanical power under the constraint of these movements. However, varying the ratio of stretch and recoil duration, power output can be modulated despite similar work (amplified or attenuated).

1.1.2.2 Power amplification

Muscle-tendon unit elasticity can serve as power amplifier by storing energy produced by the muscle in the tendon slower than releasing it. Specifically, to amplify power, mechanical energy produced by a muscle contraction is stored with a slow rate in the tendon and then released to the body with a much higher rate than it was stored. Thus, the idea of power amplification is similarly to the energy conservation concept based on the uncoupling of muscle work from joint movements, which is possible due to elastic structures. Importantly, the power output of the MTU can in this fashion be higher compared to isolated muscles, as it was demonstrated in equine biceps muscles (Wilson et al., 2003).

When performing a squat jump, high power outputs are required, whereas the available time before the jump is usually not limited. Several animals, including humans, take advantage of power amplification during jumping. The first evidence of loading of elastic tissue previous to the jump to enhance power in mammals was available from bush-babies (Aerts, 1998 for vastus) and bullfrogs (Roberts & Marsh, 2003 for plantaris muscle). A similar benefit of elastic elements to enhance squat jump performance in humans was suggested by modelling (Bobbert, 2001) and supported by *in vivo* measurements of gastrocnemius fascicle behaviour (Kurokawa et al., 2001). Gravitational loading and proximal to distal joint kinetics were identified as mechanisms to support elastic energy utilisation to amplify power (Farris et al., 2016).

1.1.2.3 Energy dissipation

When mechanical power must be absorbed, power output can also be attenuated. The involvement of tendon in energy dissipation mechanisms, has been noticed in *in vitro* preparations demonstrating that rapid stretches of the MTU were taken up by the tendon (Griffiths, 1991). Energy from the body is temporarily stored in the tendon and then released to do work on the muscle that absorbs energy by actively lengthening. Equally to the amplification of power by elastic structures, the tendon can attenuate power input to the muscle so that the MTU can absorb energy with a rate beyond the capacity of the muscle by uncoupling muscle length changes from that of the MTU.

The temporary storage of strain energy in elastic structures was demonstrated *in vivo* during landings of wild turkeys (Konow et al., 2012; Roberts & Azizi, 2010). The stretch of the tendon led to a delay of fascicle lengthening with reduced velocity. These studies suggest that the elastic buffer limits peak muscle forces, fascicle lengthening rate and fascicle power input during lengthening contractions. This was proposed to be a protective mechanism against muscle strain injuries (Roberts & Konow, 2013) due to the association of these factors with muscle damage during active muscle lengthening (Proske & Morgan, 2001). In addition, muscle strain did not increase when negative work was increased by varying the drop height, highlighting the importance of the elastic buffer (Konow & Roberts, 2015). A study investigating the interaction of muscle and elastic tissue during stair descent, indicates a similar elastic buffering mechanism in humans (Spanjaard et al., 2007).

1.1.3 The role of tendon properties for muscle-tendon interaction

Elastic elements, in their diverse roles, allow the MTU to exceed the performance of intrinsic muscle contractile properties in different ways. As highlighted by Roberts and Azizi (2011), tendon stiffness is a key parameter for the elastic mechanisms associated with muscle-tendon interaction. Indeed, it has been shown that higher tendon stiffness improves the performance in various parameters such as jump performance, running economy (Albracht & Arampatzis, 2013; Arampatzis et al., 2006; Fletcher et al., 2010) sprint performance (Stafilidis & Arampatzis, 2007) and rate of muscle force development (Bojsen-Møller et al., 2005; Waugh et al., 2013). There are conflicting results reported for endurance performance (Kubo et al., 2010b) and the direction of adaption to increase performance might depend on the muscle-tendon unit (Kubo et al., 2015).

Lichtwark and Wilson explored the influence of elasticity in the MTU for optimal power output and efficiency in a series of articles (Lichtwark & Wilson, 2005a, 2007, 2008). The results from these models highlight the decisive role of elastic properties for MTU power production and efficiency. A compliant Achilles tendon is required to achieve optimal efficiency for specific tasks depending on muscle architectural parameters and muscle activation. In their model, both reduction and increase in stiffness reduced efficiency. It is suggested that tendon mechanical properties must be tailored to their function to work effectively.

1.2 Research aims

In light of the current literature, the objective of the present thesis was to investigate the role of the Achilles tendon and its mechanical properties in the mechanical function of the human triceps surae MTU in different locomotor tasks. To examine muscle-tendon interaction during energy dissipation, we chose landing tasks, performed with body mass only and with added load. The conservation of elastic energy was studied in running at different speed and under different loading conditions. Furthermore, for both landing and running, the effect of a training-induced increase in stiffness of the Achilles tendon on muscle-tendon behaviour was investigated. The following specific research aims were examined with the respective hypotheses:

1. The first aim was to investigate muscle-tendon interaction of the human triceps surae when energy is dissipated. We predicted that during a step landing task, Achilles tendon and elastic elements would buffer the rapid stretch of the MTU to limit high muscle fascicle strains. Furthermore, we hypothesised that fascicle strain would increase with additional load to dissipate the additional energy (article one).
2. The second research objective was to examine the role of Achilles tendon properties during energy dissipation. The hypothesis was that a training-induced increase in Achilles tendon stiffness would limit the stretch of the tendon during a landing task and induce greater fascicle strain (article two).

3. The third paper aimed to study muscle-tendon interaction during running at increased speed and with additional load. We hypothesised that different strategies to increase positive work at the ankle joint would be prioritised due to differences in availability of potential energy and differences in time constraints. Utilisation of elastic strain energy would increase more with load than with increased speed (article three).
4. The aim of the fourth manuscript was to examine the effect of increased Achilles tendon stiffness on muscle-tendon interaction during running. We hypothesised that tendon strain and recoil would be reduced, which would affect fascicle behaviour, especially in soleus, during running (article four).

2 Methods

The methods used in the four manuscripts included in this thesis partly overlap and will therefore be described together as appropriate. The data for article one and three were collected during the same study, referred to hereafter as study A. The second data collection, which will be referred to as study B, provided the data for articles two and four (**Figure 4**). The protocol was approved by the ethical committee of the Norwegian School of Sport Sciences (Appendix) and the Norwegian Centre for research data approved the process of data handling (Appendix). Before participating, subjects were informed about all procedures and provided their written informed consent.

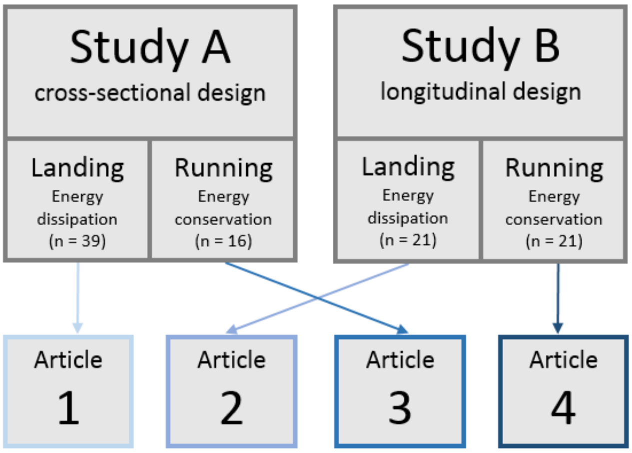


Figure 4. *Overview over the two different studies included in this thesis indicating where the data in the respective articles were derived from. n, number of subjects included.*

2.1 Subjects and experimental protocol

All tests in both studies started with a standardised warm-up including 5-min barefoot running on a treadmill at self-selected speed. Testing was then performed barefoot and measurements were taken from the right leg of the participants except EMG recordings, which were measured on the left leg.

2.1.1 Study A

A total of thirty-nine healthy male athletes participated in this study. Twenty-one of the subjects were engaged in ski jumping (age 23 ± 3 years, height 179 ± 6 cm and mass 64 ± 4 kg) and eighteen in distance running (age 27 ± 5 years, height 180 ± 5 cm and mass 68 ± 6 kg). The two different groups of athletes were originally recruited for a larger project and were merged for this study because of their homogenous anthropometrics and triceps surae muscle-tendon properties.

All subjects performed a minimum of five trials of a step landing task (see 2.3.3; article 1). We asked them to step down from a step with the right leg, while the left leg remained on the step to maintain balance. The step height was adjusted to individual body mass to standardize the potential energy (240 J). Five trials were performed to scan gastrocnemius and soleus muscle fascicles and five trials to scan gastrocnemius myotendinous junction. Electromyography activity of gastrocnemius medialis, soleus and tibialis anterior were recorded in five separate trials from the left leg because the size of the ultrasound transducer did not allow simultaneous measurements over the targeted muscles. All subjects performed the protocol twice, once without additional loading and once with a vest loaded with 20% of the individual body mass.

A subgroup of 16 runners (age 27 ± 4 years, height 179 ± 05 cm and mass 68 ± 6 kg) additionally ran under two different speed and loading conditions (see 2.3.3; article 3). The four conditions were composed of a combination of running at preferred speed and increased speed (+20% of preferred speed) and with and without additional loads (+20% of body weight). Mass-adjustable vests were used to achieve the individual loading. For each of the four conditions, ultrasonography, kinematic and kinetic data were collected for at least 10 complete step cycles. All running conditions were conducted twice to obtain ultrasound images from the muscle fascicles and the myotendinous junction.

2.1.2 Study B

Twenty-one recreationally active volunteers participated in study B. Eleven subjects were assigned to the training group (age 26 ± 4 years, height 174 ± 9 cm, mass 70 ± 9 and 69 ± 9 kg before and after training, five men and six women) and ten subjects served as controls (age 30 ± 3 years, height 178 ± 8 cm, mass 73 ± 8 kg before and after training, six men and four women). All subjects were free of musculoskeletal disorders. The training group followed a training regimen of explosive plantarflexions at neutral joint angle for 10 weeks (see 2.2). Subjects were not engaged in regular plantar flexor muscle training prior to participating in this study. The controls were asked not to change their training habits throughout the duration of the study.

We measured triceps surae muscle-tendon properties (see 2.3.1 and 2.3.2) and muscle-tendon behaviour during landing and running by the means of ultrasonography, kinematic and kinetic analysis (see 2.3.3; article 2 and 4) in the same way before and after the training period. The landing task was performed as single-leg drop landing from a height of 15 cm and subjects were asked to minimize knee flexion during the landing. To ensure good reproducibility, every subject repeated the same task five times. Running trials were conducted at preferred speed and data were collected from at least 10 step cycles. For both landing and running this protocol was conducted twice to obtain ultrasound data from fascicles and the myotendinous junction.

2.2 Training program (Study B only)

The training was conducted three times per week for 10 weeks. Each training session started with a 5-min warm-up on a cycling ergometer followed by four sets of 10 explosive unilateral plantarflexions (1s loading and 5s rest). To minimize muscle hypertrophy and strength gains while eliciting increases in Achilles tendon stiffness, we chose a short time under tension and an explosive contraction type for the training program (as shown in Massey et al., 2018 for knee extension). During the exercise, subjects were standing on the right leg in an adjustable custom-built rig with ankle, knee and hip joint in neutral position (**Figure 5**). The shoulder height of the rig was adjusted to each individual and a rigid cable with a strain gauge in series ran vertically between the shoulder arms and the ground. This prevented upward displacement of the subjects and measured forces during the plantarflexion efforts. The individual maximum force was measured during every third training with instantaneous visual feedback. Training intensity was set to 80% of the individual maximum and adjusted every week. During training, force was displayed for the subjects to match the individual target force.

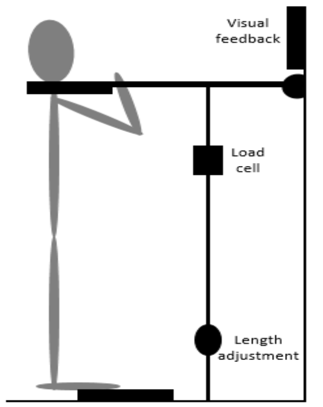


Figure 5. *Height adjustable training rig for isometric plantarflexions providing visual feedback of the exerted force.*

2.3 Measurements and data processing

2.3.1 Muscle strength and architecture

We measured plantarflexion strength while subjects were lying prone on a dynamometer (Isomed 2000 D. & R. Ferstl GmbH, Hemau, Germany) with hip, knee and ankle joints securely fixed at anatomical positions. Straps and stiff pads were adjusted to minimize trunk, hip, knee and ankle movement. After a specific warm-up consisting of at least five submaximal plantar flexions, maximum plantar flexion torque was determined as the higher of two maximal voluntary contractions.

Ultrasound images of resting muscle architecture were taken in the same body position from the mid-belly of the gastrocnemius medialis muscle (HL9.0/60/128Z-2, LS 128 Telemed, Vilnius, Lithuania). We used a software for image analysis (ImageJ, National Institutes of Health, Bethesda, MD, United States) to measure muscle fascicle length, pennation angle and thickness of gastrocnemius medialis. Fascicle length was defined as straight line between the superficial and deep aponeurosis, running parallel to the visible portions of the fascicles. In a few cases, where fascicle length exceeded the field of view, linear extrapolation was used. Pennation angle was measured as the angle between the segmented fascicle and the orientation of the deep aponeurosis. The average of the shortest distance between the two aponeurosis measured at 25, 50 and 75% of the field of view width, was defined as muscle thickness.

2.3.2 Achilles tendon stiffness

Subjects were asked to perform five ramp contractions up to 90% of the individual maximal torque to estimate Achilles tendon stiffness. They were asked to increase the torque with a rate of 100 $n \cdot s^{-1}$, which was displayed on a screen in front of the subjects during all trials. Prior to recording the trails, the subjects practiced increasing the torque at the constant rate to become familiarised to the task and to precondition the tendon (Maganaris, 2003b).

During the ramp contractions, ultrasound scans (80 Hz) of the gastrocnemius myotendinous junction, plantarflexion torque (600 Hz) and kinematic marker trajectories (120 Hz) were recorded simultaneously. Data were synchronised by an analog trigger signal sent by the ultrasound device. The ultrasound transducer was secured with self-adhesive tape over the gastrocnemius medialis myotendinous junction. To allow consistent scanning when the muscle bulges, we placed a gel pad between the transducer and the skin. Four motion analysis cameras (Qualisys, Gothenburg, Sweden) captured the trajectories of three reflective markers, which were rigidly attached to a cast fitting the ultrasound transducer, and one marker placed over the tendon insertion on the calcaneus. Marker trajectories and plantarflexion torque data were filtered using a second order zero-phase digital low-pass Butterworth filter with a cut-off frequency of 15 Hz. The measured joint torque was corrected for inevitable joint movement by an inverse kinetic approach using the position of kinematic markers on the medial malleolus and the footplate of the dynamometer (Arampatzis et al., 2005). The torque contribution of the triceps surae to plantarflexion torque was set to 91%, which has previously been estimated from normative data (Dick et al., 2016). Achilles tendon force was calculated by dividing the corrected plantarflexion torque by the internal moment arm of the Achilles tendon. The moment arm was defined as the mean perpendicular distance from the tendon to the midpoint between the medial and lateral malleolus measured externally using a tape measure.

The position of the gastrocnemius myotendinous junction was determined by tracking the closest visible fascicle insertion offline using a semi-automatic tracking algorithm (Tracker 4.95, physlets.org/tracker/). Ultrasound data were filtered using a similar filter as for marker trajectories with a cut-off frequency of 6 Hz. The position of the ultrasound image relative to the kinematic markers was established by a prior calibration to calculate the position of the myotendinous junction in the laboratory coordinate system. Subsequently, Achilles tendon length was defined as the straight distance between the myotendinous junction and the calcaneus marker over the tendon insertion.

Achilles tendon stiffness was then calculated for every subject during each of the two test sessions from the third-order polynomial fitted mean force-elongation relationship between 50% and 80% of the maximum individual force of three trials. From the recorded five trials, the trials yielding the lowest and highest stiffness values were excluded. Measurement of tendon strain were obtained at 80% of the maximum force produced common in both test sessions.

2.3.3 Measurements during dynamic tasks

All data collected during the landing tasks and during running were synchronised using the trigger signal of the ultrasound device.

2.3.3.1 Joint mechanics

Kinematics of the right leg were recorded using an infrared camera system (Qualisys, Gothenburg, Sweden; 12-15 cameras) operating at 300 Hz. Twenty retroreflective markers were placed according to a modified Cleveland Clinics marker set. Joint centres and segments were defined during a static calibration trial. The pelvis segment and the hip joint centres were determined using markers on the left and right anterior and posterior iliac spines (Bell et al., 1989). The mid-distances between two markers on the medial and lateral condyles and between the medial and lateral malleoli were determined as knee and ankle joint centre, respectively. Markers on the calcaneus and the first, second and fifth metatarsal defined the foot as one segment. Additional tracking markers were placed on the lateral side of the right thigh and shank as clusters consisting of four markers each. Data were filtered with a bidirectional first or second order (landing and running, respectively) low-pass Butterworth filter with a 15 Hz cut-off frequency. Joint angles were calculated between the two respective segments of interest using the coordinate system of the proximal segment as reference (Visual 3D, C-Motion Inc., Germantown, MD, USA). We estimated gastrocnemius and soleus MTU length based on frequently used regression equations (Hawkins & Hull, 1990) using ankle and knee joint angle data in combination with shank length. Shank length was defined as the distance between the lateral epicondyle and the lateral malleolus.

All trials were conducted on force plates instrumented to a treadmill (M-Gait, Motekforce Link, Amsterdam, The Netherlands). Force data were recorded at a frequency of 1500 Hz and filtered similar to the marker trajectories. Ankle and knee joint moments were computed in Visual 3D, where the moment was resolved in the coordinate system of the segment proximal to the joint. Joint power was calculated as the product of the joint moment and the angular velocity of the joint.

2.3.3.2 Fascicle and tendon length measurements

Ultrasonography images were collected at 80 Hz in two different trials to firstly examine gastrocnemius and soleus fascicle length and pennation angle and secondly to determine the position of the gastrocnemius myotendinous junction to calculate Achilles tendon length during the landing and running tasks. Self-adhesive tape and a cast fitted to the transducer prevented movement of the transducer during all trials. To ensure the scanning quality when the muscle was bulging, we placed an ultrasound gel pad between the transducer and the skin during scans of the myotendinous junction.

The flat shaped ultrasound transducer was placed over the gastrocnemius muscle belly so that soleus fascicles could be imaged simultaneously. The reliability of ultrasonography to measure fascicle length has previously been shown (Gillett et al., 2013; Kwah et al., 2013) and ensured by the controlled design of Study B. A semi-automated tracking algorithm was used to analyse fascicle length and pennation angle in the ultrasound images (Cronin et al., 2011; Farris & Lichtwark, 2016). Fascicles were defined parallel to the visible collagenous structures between the deep and superficial aponeurosis. Pennation angle was measured as the angle between the fascicle and the deep aponeurosis for gastrocnemius and the superficial aponeurosis for soleus, which was tracked at the same time.

Achilles tendon length was estimated using the same approach as described earlier during isometric contractions (2.3.2). The vector representing the tendon was then used to calculate the instantaneous Achilles tendon moment arm as the shortest perpendicular distance from the tendon to the ankle joint centre defined as the midpoint between the two malleoli marker (Obst et al., 2017). The force of the whole Achilles tendon was subsequently estimated by dividing ankle joint moment by the calculated tendon moment arm at each time point. For the running data in study B we used a constant, externally measured moment arm (as during isometric contractions).

Muscle length changes were estimated as the projected fascicle length in the line of action of the muscle (fascicle length was multiplied with the cosine of pennation angle) to calculate the architectural gear ratio of gastrocnemius and soleus during stance (AGRs). The ratio was defined as muscle strain divided by fascicle strain (Brainerd & Azizi, 2005) during the stance phase of running (article 4 only, the calculation was similar to Hollville et al. (2018)). The length of elastic elements was estimated by subtracting the vertically projected fascicle length from the MTU length (Fukunaga et al., 2001) during landing (article 1 only).

2.3.3.3 Measurements of muscle activity (Study A only)

Electromyographic (EMG) activity of gastrocnemius, soleus and tibialis anterior were recorded from the left leg (TeleMyo DTS, Noraxon U.S.A. Inc., Scottsdale, AZ, USA) at a frequency of 1500 Hz. Electrode placement and preparation of the measurement sights were implemented according to SENIAM guidelines (Hermens et al., 2000). Data were first filtered using a bidirectional high-pass Butterworth filter with a 20 Hz cut-off, followed by rectifying of the EMG signal. Subsequently, data were treated with a 6 Hz bidirectional low-pass Butterworth filter. For each individual, EMG data were normalised to the peak values during the landing task and during running without loading at preferred speed (for running).

2.4 Data reduction and statistics

All data were resampled to 101 data points over the phase of negative ankle power for landing or over the stride cycle and stance phase duration for running. The landing phase was further divided into three sub-phases based on the GRF, where phase one was characterised as nearly constant force, phase two was defined by a force rise and phase 3 by force decay. For EMG data during landing the phase of negative power could not be identified because kinematic data were not available. Therefore, matching events of the GRF with the right leg were used to define the landing phase. Velocities of MTU fascicles and tendon were determined by differentiating their length with respect to time. Negative and positive ankle and knee joint work were calculated by integrating the negative and positive power produced during the stance phase using the trapezoidal integration method. Individual joint net-work was calculated as the differences between negative and positive work. Mean values for each subject were calculated after removing single deviating trials so that four to five trials were used for the landing task and eight to ten steps for the running in both studies. We had to discard some data due to insufficient quality (Study A: soleus ultrasound data for two subjects and kinematics data in the in the added mass condition for two other subjects during landing; Study B: soleus fascicle data for three subjects of the training group and myotendinous junction ultrasound data for two subjects of the training group and three subject of the control group during running). Finally, group mean values were calculated.

For statistical analysis we used repeated measures one-way (article 1) and two-way ANOVA (article 2-4). Tukey and Sidak multiple comparisons were performed, when interaction or main effects were found. Factors in the ANOVA design for the different tasks were “phase” for the landing in Study A, “load” and “speed” for the running in Study A and “time” and “group” for the landing and running in Study B. Results are presented as mean ± standard deviation in the text, tables and error-bar plots and as mean ± standard error of the mean in the line figures to illustrate the precision of the mean.

3 Results

3.1 Study A

3.1.1 Landing (Article 1)

3.1.1.1 Joint kinematics and kinetics

During the single-leg step landing task, the ankle was in a plantar flexed position at touch-down and subjects performed a dorsiflexion during the subsequent landing. The knee flexed during the landing after being in a relatively neutral angle at touch-down. Ankle joint peak moment and power were higher compared to the knee joint and the peaks occurred earlier during the landing phase (**Figure 6 A, D, G**). With loading, the landing duration was prolonged by 10%. Joint flexion was greater and peak moment and power were increased at ankle and knee (**Figure 6 B-C, E-F, H-I**).

3.1.1.2 Muscle-tendon measures

Gastrocnemius and soleus MTU lengthened throughout the landing period, whereas the highest lengthening velocities were reached during phase two (539 ± 155 $mm \cdot s^{-1}$ for gastrocnemius and 545 ± 145 $mm \cdot s^{-1}$ for soleus; **Figure 7A, C**). During phase three, soleus MTU lengthened significantly more than gastrocnemius MTU ($P \leq 0.01$). In contrast, fascicles shortened during the first phase and lengthened during phase two and three. Gastrocnemius strains were greater in all three phases compared to soleus ($P \leq 0.01$) and gastrocnemius fascicles lengthened at higher velocities than soleus fascicles (214 ± 50 $mm \cdot s^{-1}$ for gastrocnemius and 132 ± 32 $mm \cdot s^{-1}$ for soleus; **Figure 7 B, D**).

Achilles tendon and elastic elements lengthened during phase one and two. During phase three, Achilles tendon length did not change but elastic elements shortened significantly. During phase two, when negative power production and tendon lengthening was highest, Achilles tendon lengthening was significantly lower than lengthening of the combined elastic elements (2.9 and 3.6%, respectively; **Figure 7 E, F**).

When subjects were loaded during the step landing, gastrocnemius and soleus MTU lengthening was larger during phase two and three. Similarly, Achilles tendon stretch was larger during phase two, whereas elastic elements differed between the loading conditions during phase one and two. Loading did not alter gastrocnemius or soleus muscle fascicle length (**Figure 8**).

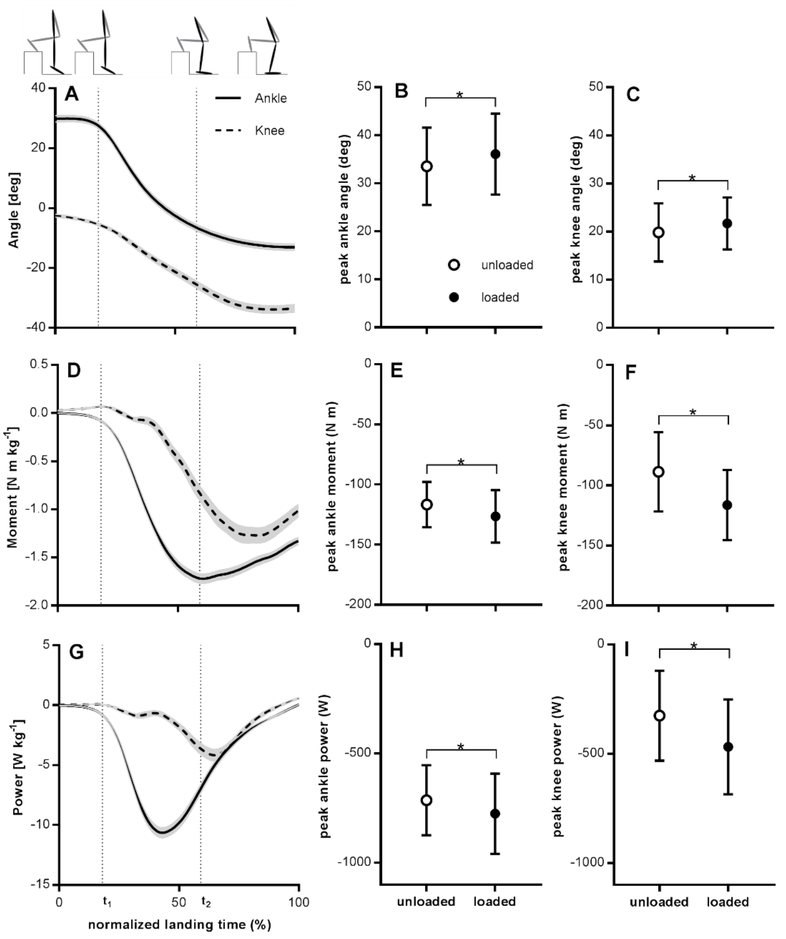


Figure 6. *Joint angle, moment and power at ankle and knee joint during energy absorption in the unloaded condition (A, D, G). Data are means ± s.e.m. and time series are normalised to 101 points. Moment and power were normalised to body mass. Vertical dotted lines represent the time points of the highest rate of GRF force development (t_1) and peak GRF (t_2) and are referred to as phase 1-3 in the text. Peak values were compared between the unloaded and loaded (+20%) conditions for ankle and knee angle (B and C), moment (E and F) and power (H and I). Data are means ± s.d., *$P \leq 0.05$ when compared with the other condition.*

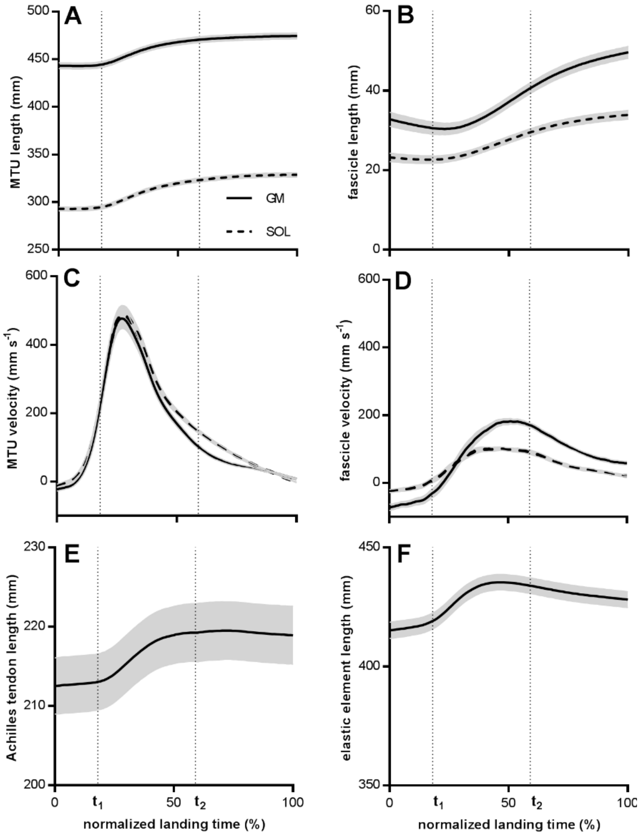


Figure 7. *Gastrocnemius medialis (GM) and soleus (SOL) muscle-tendon unit length (MTU) and fascicle length (A and B) and their velocities (C and D), and length of the Achilles tendon and elastic elements (E and F) during energy absorption. Data are means ± s.e.m. and time series are normalised to 101 points. Vertical dotted lines represent the time points of the highest rate of GRF force development (t_1) and peak GRF (t_2) and are referred to as phase 1-3 in the text.*

Table 1. *Mean length changes relative to the length at t_0 of Achilles tendon, elastic elements, and gastrocnemius medialis and soleus muscle-tendon unit and fascicles at selected time points (t_1-t_3) in the unloaded condition.*

	ΔL_{MTU} (mm)		**ΔL_f (mm)**			
Time point	**GM**	**SOL [mm]**	**GM**	**SOL**	**ΔL_{AT} (mm)**	**ΔL_{EE} (mm)**
t_1	1.3 ± 2.1*	1.9 ± 2.1*	-2.3 ± 2.2*	-0.5 ± 1.2*	0.5 ± 1.0*	3.9 ± 2.6*
t_2	27.4 ± 7.4*	30.2 ± 7.3*	7.6 ± 6.9*	6.3 ± 3.5*	6.7 ± 4.4*	18.7 ± 7.9*
t_3	31.3 ± 7.2*	35.6 ± 7.2*	16.8 ± 6.6*	10.6 ± 3.2*	6.4 ± 4.2	12.9 ± 7.4*

Data are means ± s.d.; L_{MTU}, muscle-tendon unit length; L_f, fascicle length; L_{AT}, Achilles tendon length; L_{EE}, elastic element length; GM, gastrocnemius medialis; SOL, soleus. t_0, onset of negative ankle power; t_1, highest rate of ground reaction force development; t_2, peak ground reaction force; t_3, end of negative ankle power. *$P < 0.05$ when compared to the preceding event.

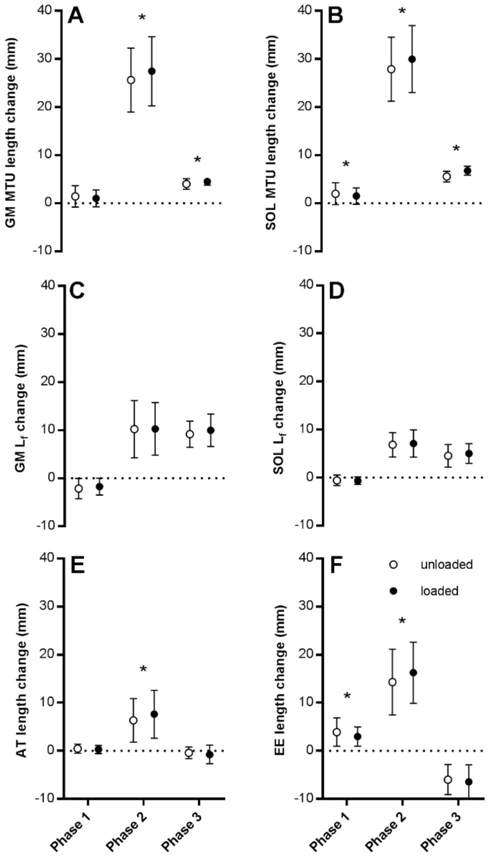


Figure 8. _Length changes of the muscle-tendon unit (MTU) and its components fascicles (f), Achilles tendon (AT) and elastic elements (EE) during the three different phases of the landing task unloaded and loaded. Data are means ± s.d.; Phases are based on GRF with phase one defined as small increase in force, phase two as force rise, and phase three as force decay. *P≤0.05 when compared with the other condition._

3.1.1.3 Muscle activity

EMG signals from gastrocnemius, soleus and tibialis anterior were normalised to their respective peak values obtained during the landing phase when executing the task without loading. The greatest activity of gastrocnemius occurred during the first phase. Soleus activity increased throughout phase one and two. Loading increased soleus and tibialis activity but not that of gastrocnemius (**Figure 9**).

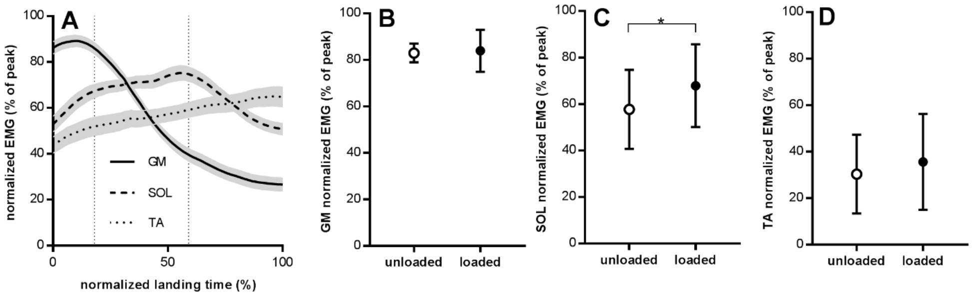


***Figure 9**. Electromyographic (EMG) activity of gastrocnemius medialis (GM), soleus (SOL), and tibialis anterior (TA) during energy absorption in the body mass condition (A). Data are means ± s.e.m. for the line plot; Time series are normalised to 101 points; Vertical dotted lines represent the time points of the highest rate of GRF force development (t_1) and peak GRF (t_2) and are referred to as phase 1-3 in the text. Comparison of peak EMG activity during energy absorption unloaded (unfilled circles) and loaded (filled circles) (B-D). Data are means ± s.d.; All data are normalised to the peak values during the step-landing task. *P≤0.05 when compared with the other condition.*

3.1.2 Running (Article 3)

3.1.2.1 Running kinematics and kinetics

The mean preferred running speed was 3.1 ± 0.3 m·s^{-1} and the increased running speed was 3.7 ± 0.3 m·s^{-1}. The duration of a step cycle and the stance duration decreased when speed was increased in both the unloaded and loaded condition. When load was added, gait cycle duration was reduced at increased speed (-3%) but not at preferred speed. In contrast, stance duration was longer with loading at either speed (**Table 2**).

Ankle joint positive work was higher when speed and load were increased. The increase in positive work at the ankle was consistent at both loading and speed conditions. More positive work was also done by the knee joint with loading compared to unloaded running at either speed but the increase in speed did not affect knee joint work (**Table 2**). Joint kinematics were consistent across speed and loading conditions evidenced by similar peak ankle and knee joint angles.

3.1.2.2 Muscle-tendon measures

Accordingly, MTU peak lengths and the extent of MTU shortening were similar across speed and loading conditions (**Figure 10 A, B**). Yet, with higher speed, maximum MTU shortening velocity was increased at both loading conditions. Loading reduced MTU velocities but post-hoc tests revealed that this was only significant at preferred speed (**Table 2**).

Gastrocnemius and soleus fascicle length were not affected by either speed or loading across speed and loading modalities (**Figure 10 C, D**) evidenced by similar mean fascicle length during stance, fascicle shortening amplitude and peak fascicle shortening velocity across conditions (**Table 2**).

Achilles tendon stretch and peak length were not statistically affected by speed or load, whereas a trend towards a higher lengthening velocity was observed ($P = 0.06$). In contrast, tendon recoil amplitude increased with loading at both speeds but did not change when running speed increased (**Figure 10 E**). There was an interaction effect of speed and loading for tendon recoil velocity. Similar to recoil amplitude, velocity was increased with loading at either speed. When speed was increased, recoil velocity increased when running with load but not when running unloaded (**Table 2**). Speed and loading caused an increase in the calculated peak Achilles tendon force at both modalities, respectively (**Table 2**).

Table 2. *Kinematic and kinetic parameters during unloaded and loaded running at preferred and increased speed obtained during the stance phase.*

			Unloaded		**Loaded**	
			Preferred speed	**Increased speed**	**Preferred speed**	**Increased speed**
Duration [s]		**Cycle**	0.68 ± 0.04	0.66 ± 0.05§	0.68 ± 0.05	0.64 ± 0.05*§
		Stance	0.23 ± 0.02	0.21 ± 0.02§	0.26 ± 0.02*	0.23 ± 0.02*§
Work [J]	**positive**	**Ankle**	55 ± 13	62 ± 15§	62 ± 18*	69 ± 20*§
		Knee	14 ± 8	16 ± 10	17 ± 9*	19 ± 10*
	negative	**Ankle**	32 ± 14	36 ± 16§	37 ± 18*	43 ± 20*§
		Knee	27 ± 11	29 ± 12	31 ± 11*	31 ± 11
	net	**Ankle**	23 ± 6	26 ± 7	25 ± 10	26 ± 11
		Knee	-13 ± 7	-13 ± 5	-14 ± 9	-12 ± 6
Peak shortening velocity [mms^{-1}]		**AT**	185 ± 42	199 ± 42	213 ± 60*	283 ± 77*§
		GM MTU	689 ± 118	779 ± 96§	607 ± 139*	765 ± 111§
		SOL MTU	656 ± 120	760 ± 103§	570 ± 128*	727 ± 99§
		GM fasc.	130 ± 60	133 ± 46	141 ± 73	147 ± 72
		SOL fasc.	115 ± 49	125 ± 51	106 ± 34	123 ± 43
Force [N]		**AT**	4336 ± 931	4644 ± 1037§	4501 ± 1029*	4896 ± 1059*§
Impulse [Ns]			469 ± 59	452 ± 61§	560 ± 76*	529 ± 75*§

Values are means ± sd; GM, gastrocnemius medialis; SOL, soleus; AT, Achilles tendon; MTU, muscle-tendon unit; fasc., fascicle. *Significantly different from the unloaded condition at the same speed; §Significantly different from the preferred speed condition with the same load.

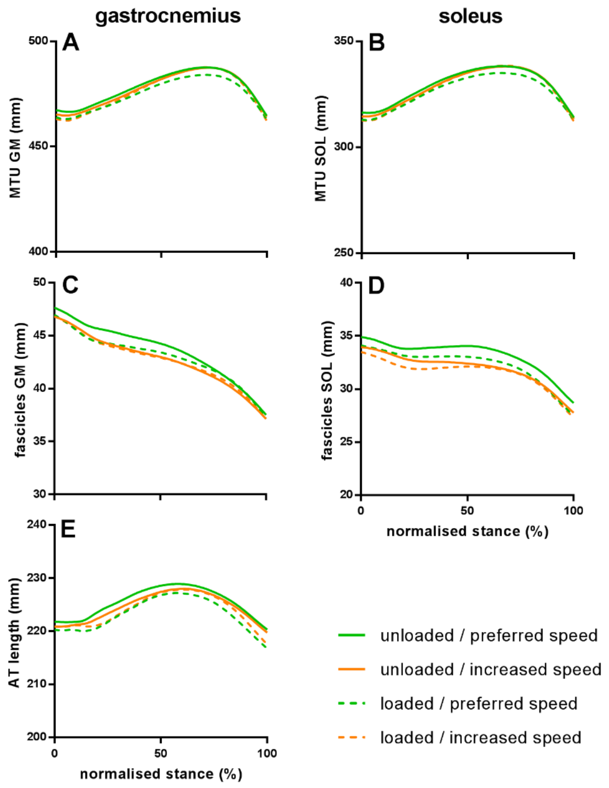


Figure 10. *Gastrocnemius medialis (GM) and soleus (SOL) muscle-tendon unit (MTU) length (A and B), fascicle length (C and D) and length of the Achilles tendon (E) during the stance phase of running. Data are group means and time series are normalised to 101 points. Line colour indicates the different speed conditions and line style indicates different loading conditions.*

3.1.2.3 Muscle activity

There was an interaction effect of speed and load on gastrocnemius and soleus muscle activity. Running at higher speed increased the mean activity during stance of both muscles, regardless of the loading modality. Loading depressed the EMG activity when running at preferred speed and did not change activity at increased speed (**Figure 11 B, A**). Mean EMG activity of the antagonist tibialis anterior during the stance phase increased with speed in both loading conditions. In contrast, loading did not affect tibialis anterior activity (**Figure 11 C**). Overall, tibialis activity was highest during the swing phase in all conditions, whereas gastrocnemius and soleus peaked during stance.

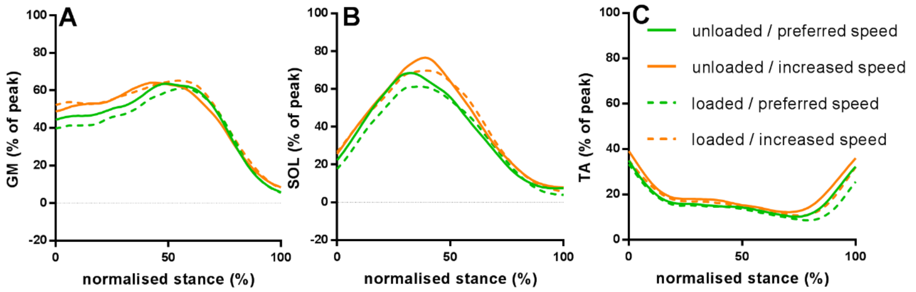


Figure 11. *Electromyographic (EMG) activity of gastrocnemius medialis (GM), soleus (SOL), and tibialis anterior (TA) during energy absorption in the body mass condition (A). Data are means ± s.e.m.; Time series are normalised to 101 points. Line colour indicates the different speed conditions and line style indicates different loading conditions.*

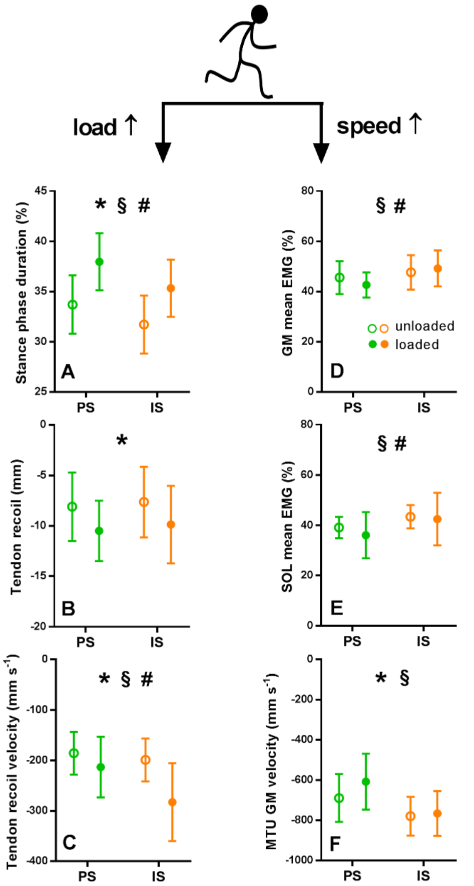


Figure 12. *Key variables during the stance phase that vary with loading (unfilled circles – unloaded, filled circles – loaded) or speed (preferred speed (PS) compared to increased speed (IS)): stance phase duration (A), Achilles tendon shortening (B), Achilles tendon shortening velocity (C), gastrocnemius medialis (GM) EMG activity (D), soleus (SOL) EMG activity and muscle-tendon unit (MTU) activity (F). Data are means ± s.d., # P<0.05 for interaction effect, *P<0.05 for main effects of mass, and § P<0.05 for main effect of speed.*

3.2 Study B

3.2.1 Effect of training on muscle-tendon properties (Articles 2 and 4)

The average increase in plantarflexion strength was 15% after the training intervention (**Table 3**) while the one repetition maximum during the training exercise was increased by 39% (from 1670 ± 393 N in week one to 2317 ± 607 N in week 10). A concomitant 5% increase was measured for gastrocnemius medialis muscle thickness and pennation angle, whereas fascicle length did not change. Achilles tendon stiffness increased by 18% in the training group, however the 9% reduction in tendon strain at individual maximal force during isometric contractions was not significant. None of the variables changed significantly in the control group. Mean values of training group and controls for all parameters are presented in **Table 3**.

Table 3*. Plantarflexion strength, resting muscle architecture and Achilles tendon stiffness during pre-intervention tests and post-intervention tests in training and control group.*

	Training group		**Control group**	
	Pre	**Post**	**Pre**	**Post**
Torque [N m] §	172 ± 50	198 ± 51 *	170 ± 51	180 ± 62
L_f GM [mm]	89.2 ± 133	90.7 ± 161	84.2 ± 161	84.0 ± 104
PA GM [°]	18.1 ± 1.8	19.0 ± 2.0 *	18.4 ± 1.1	18.3 ± 1.7
Thickness GM [mm] §	23.7 ± 35	25.0 ± 38 *	23.1 ± 38	23.5 ± 36
AT stiffness [N mm-1] †§	397 ± 146	459 ± 147 *	399 ± 193	400 ± 212
AT strain [mm]	4.4 ± 1.1	4.1 ± 0.5	4.0 ± 1.5	3.9 ± 1.5

Values are means ± sd; GM, gastrocnemius medialis; SOL, soleus; L_f, length of fascicle; PA, pennation angle; † $P < 0.05$ interaction effect; § $P < 0.05$ main effect of time; * $P < 0.05$ comparing pre- and post-intervention test.

3.2.2 Landing (Article 2)

3.2.2.1 Joint kinematics and kinetics during landing

Ankle angle at touch-down was less plantar flexed in the training group after training ($P = 0.03$) and control subjects landed in a less plantar flexed position than the trained subjects ($P < 0.01$). Similar landing duration, peak GRF, Achilles tendon force and knee and ankle joint moment showed that subjects in both groups performed the landing task in the same way during both testing sessions apart from the difference in ankle joint angle at touch-down.

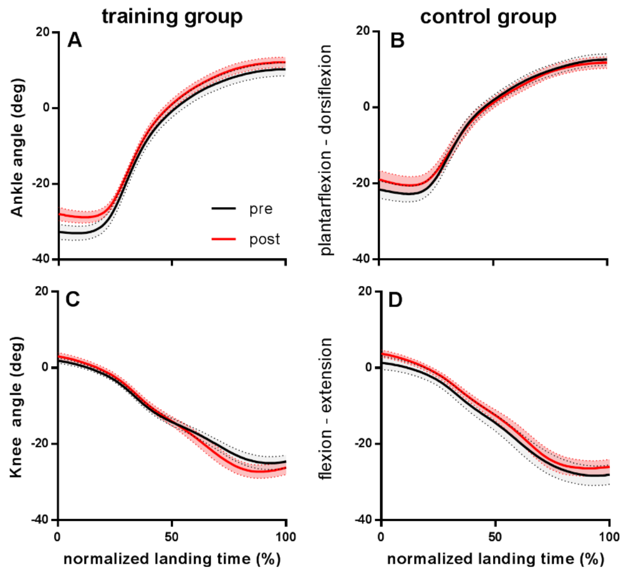


Figure 13. *Joint angles of ankle (A and B) and knee joint in the training group and the control group during energy absorption before (pre) and after (post) the training intervention. Data are means ± s.e.m. and time series are normalised to 101 points.*

Table 4. *Peak ankle and knee moment and power, Achilles tendon force, muscle-tendon unit work and landing duration in the training group and the control group measured in the pre-intervention test and the post-intervention test.*

	Training group		**Control group**	
	pre	**post**	**pre**	**post**
Ankle moment [N m]	-143 ± 34	-141 ± 30	-153 ± 26	-154 ± 34
Ankle power [W]	-976 ± 260	-1012 ± 186	-1027 ± 192	-998 ± 193
Ankle work [J]	-78 ± 25	-70 ± 30	-74 ± 21	-69 ± 13
Knee moment [N m]	90 ± 45	108 ± 42	117 ± 45	112 ± 40
Knee power [W]	-314 ± 203	-455 ± 218	-549 ± 245	-532 ± 312
Knee work [J]	-16 ± 16	-19 ±13	-27 ± 15	-20 ± 16
AT force [N]	2746 ± 555	2750 ± 518	2848 ± 493	2857 ± 736
MTU work [J]	40 ± 13	39 ± 10	34 ± 8	30 ± 7
Duration [s]	0.205 ± 0.014	0.202 ± 0.013	0.204 ± 0.020	0.202 ± 0.020

Values are means ± sd; AT, Achilles tendon; MTU, muscle-tendon unit. No statistical differences were observed between pre and post-test.

3.2.2.2 Muscle-tendon measures during landing

The group mean values for gastrocnemius and soleus MTU length and their components are presented in **Figure 14** for the training group and the control group before and after the training period. Both MTUs lengthened throughout the landing after a short quasi-isometric phase at the beginning of the landing. Matching the differences in joint angle at touch-down, gastrocnemius – but not soleus- MTU length was longer at touch-down, but *post-hoc* comparisons did not reveal differences in either group (**Table 5**).

By contrast, muscle fascicles shortened after touch-down ($P < 0.01$) and lengthened throughout the rest of the landing ($P < 0.01$). Gastrocnemius fascicles were longer at touch-down after the training intervention ($P = 0.01$) but fascicle length did not change between tests for the controls ($P = 0.93$) and for neither group for soleus fascicles ($P = 0.69$). The onset of fascicle lengthening occurred later after the training intervention (7% for gastrocnemius ($P < 0.01$) and 8% for soleus ($P = 0.01$)), whereas it did not change in the controls ($P = 0.79$ and $P = 0.61$, respectively). Training also had a significant effect on fascicle lengthening. Both gastrocnemius and soleus fascicle lengthening magnitude were reduced in the training group. Peak and mean shortening velocity of gastrocnemius were reduced accordingly, while this reduction did not reach significance for soleus (**Table 5**, **Figure 15 C, D**). To ensure that the observed differences were not due to the slight differences in task execution, all statistical analysis of fascicle data was performed with values normalised to instantaneous MTU length confirming the results (**Figure 15 A, B**). Achilles tendon stretched in the first half of the landing and remained at constant length in the second half. There were no differences in tendon length at touch-down ($P = 0.71$).

Table 5. *Peak length changes and velocities of gastrocnemius medialis and soleus muscle-tendon unit, fascicles, Achilles tendon and muscle during the landing task in training and control group measured in the pre-intervention test and the post-intervention test.*

		Training group		**Control group**	
		pre	**post**	**pre**	**post**
Δl	**MTU GM †**	-32 ± 7	-30 ± 6	-25 ± 6	-23 ± 6
Max	**MTU SOL**	-35 ± 7	-33 ± 5	-30 ± 6	-27 ± 6
lengthening	**fascicles GM †**	-14 ± 4	-10 ± 2*§	-16 ± 3	-15 ± 2
[mm]	**fascicles SOL**	-11 ± 4	-8 ± 2*	-12 ± 3	-11 ± 2
	AT GM	-12 ± 2	-11 ± 3	-10 ± 3	-11 ± 3
	muscle GM	-23 ± 7	-22 ± 6	-18 ± 7	-15 ± 7
peak vel	**MTU GM**	617 ± 163	560 ± 118	508 ± 131	451 ± 132
[mm s^{-1}]	**MTU SOL**	610 ± 151	567 ± 100	514 ± 115	467 ± 114
	fascicles GM	134 ± 44	106 ± 26*§	155 ± 24	148 ± 24
	fascicles SOL	102 ± 26	91 ± 17	118 ± 32	112 ± 19
	AT GM	265 ± 36	250 ± 58	264 ± 73	297 ± 88

Values are means ± sd; GM, gastrocnemius medialis; SOL, soleus; * $P < 0.05$ comparing raw values pre- and post-test, § $P < 0.05$ comparing values normalised to instantaneous MTU length pre and post-test, † $P < 0.05$ comparing baseline, raw values between the groups. NB: When normalised to MTU length, all variables were similar between groups at baseline.

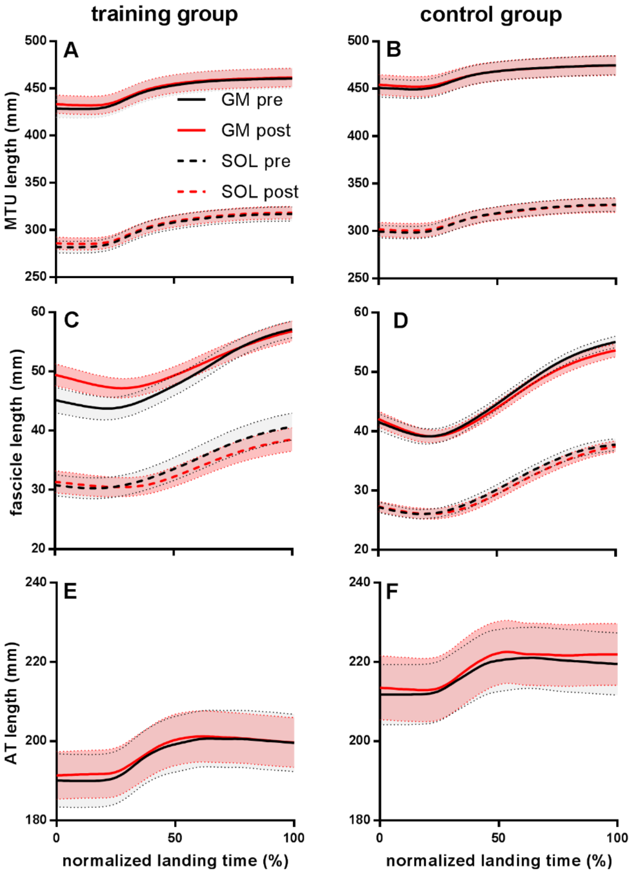


Figure 14. *Gastrocnemius medialis (GM) and soleus (SOL) muscle-tendon unit (MTU) length (A and B), fascicle length (C and D) and Achilles tendon length (E and F) during energy absorption. Data are means ± s.e.m. and time series are normalised to 101 points.*

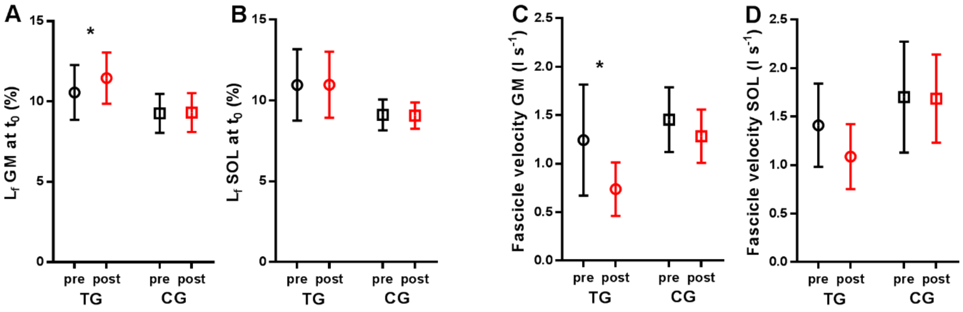


Figure 15*. Comparison of gastrocnemius medialis (GM) and soleus (SOL) fascicle length at touch-down (A and B) and mean fascicle velocity (C and D) in the training group (TG, circles) and the control group (CG, squares) between the two test sessions. Data are means ± s.d.; Fascicle length data at touch-down (A and B) are normalised to instantaneous muscle-tendon unit lengths.*P≤0.05 when compared with the other condition.*

3.2.3 Running (Article 4)

3.2.3.1 Joint kinematics and kinetics

There were no differences in running kinematics and kinetics between the two test sessions for training or control group indicated by similar changes in ankle and knee joint angles and peak joint moments (**Figure 16**). However, while knee flexion and extension were similar after training (P = 0.99 and P = 0.77), there was a trend towards increased dorsiflexion (P = 0.08) and subsequent plantarflexion (P = 0.21) in the training group.

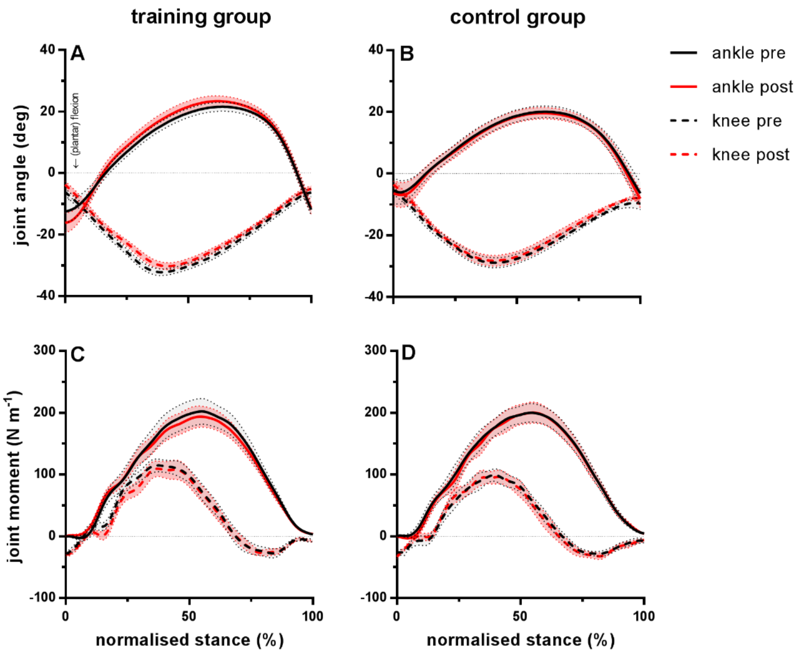


Figure 16. Ankle and knee joint angles (A and B) and moments (C and D) during the stance phase of running at preferred speed for the training group and the control group. Data are means ± s.e.m. and time series are normalised to 101 points.

3.2.3.2 Muscle-tendon measures

As joint angles indicate, the typical MTU stretch and shortening amplitudes during running were similar between the two test sessions for gastrocnemius (Interaction for stretch P = 0.11 and shortening P = 0.25) and soleus (Interaction P = 0.11 and P = 0.76, respectively) (**Figure 17 A, B**). We did not find a difference between tests for stretch of the Achilles tendon

(Interaction $P = 0.69$) but tendon shortening was reduced in the training group (Interaction $P = 0.02$, Post-test $P < 0.01$) (**Table 6**; **Figure 17 E, F; Figure 18 A, B**).

Fascicle shortening amplitude and velocity during the stance phase did not differ after training for either muscle (**Table 6**; **Figure 17 C, D; Figure 18 C, D**). The change in pennation angle during stance was increased in gastrocnemius (Interaction $P = 0.10$, Time $P = 0.04$, Post-test $P = 0.01$) and correspondingly AGRs was also increased after training (Time $P = 0.05$, Post-test $P = 0.01$). However, soleus change in pennation angle and AGRs did not differ (no significant interaction or time effect) (**Table 6**, **Figure 19**).

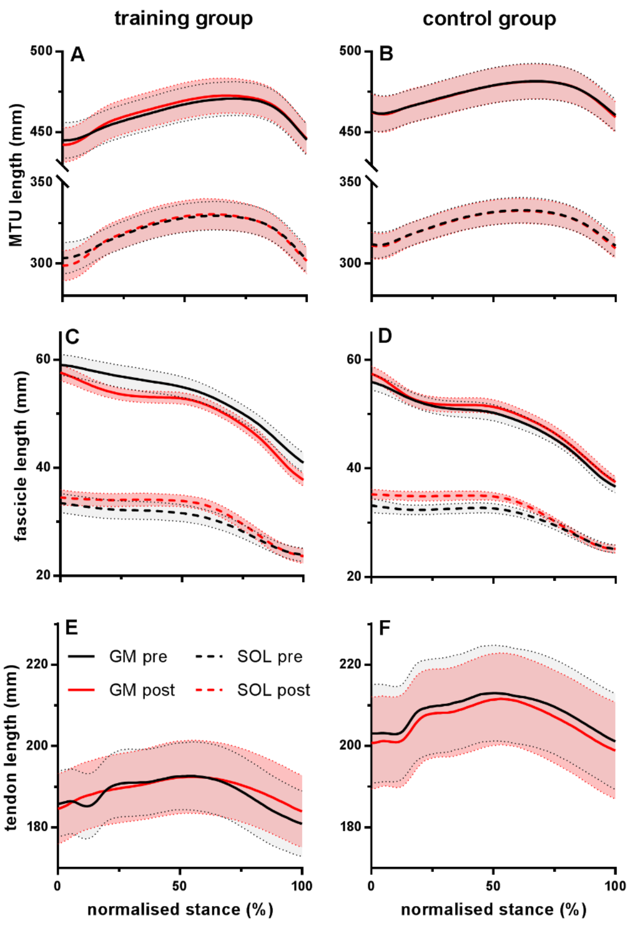


Figure 17. *Muscle-tendon unit (MTU) (A and B), fascicle (C and D) and tendon (E and F) length in the training group and the control group for gastrocnemius medialis (GM) and soleus (SOL) before (pre) and after (post) the training intervention during the stance phase of running. Data are means ± s.e.m. and time series are normalised to 101 points.*

Table 6. *Relevant variables for muscle-tendon unit (MTU), Achilles tendon (AT), and fascicles (F) of gastrocnemius (GM) and soleus (SOL) behaviour during the stance phase of running at preferred speed. Data are presented for the training group and the control group before (pre) and after (post) the tendon stiffening training intervention.*

		training group		**control group**	
		pre	**post**	**pre**	**post**
Stretch (mm)	**MTU GM**	26 ± 10	30 ± 9	19 ± 7	19 ± 7
	MTU SOL	26 ± 10	32 ± 9	21 ± 7	22 ± 7
	AT	8 ± 3	8 ± 2	10 ± 3	11 ± 4
Shortening (mm)	**MTU GM**	26 ± 4	27 ± 7	21 ± 8	22 ± 7
	MTU SOL	27 ± 4	29 ± 8	22 ± 9	23 ± 7
	AT [a]	13 ± 2	9 ± 4 [c]	10 ± 3	10 ± 3
	F GM	18 ± 5	20 ± 2	19 ± 5	20 ± 4
	F SOL	10 ± 3	11 ± 3	8 ± 3	10 ± 2
Shortening vel (mm s^{-1})	**F GM**	179 ± 51	205 ± 41	163 ± 48	166 ± 17
	F SOL	108 ± 41	133 ± 41	95 ± 22	114 ± 17
AGRs	**GM** [b]	1.06 ± 0.03	1.09 ± 0.03 [c]	1.07 ± 0.03	1.08 ± 0.02
	SOL	1.06 ± 0.11	1.05 ± 0.11	1.12 ± 0.09	1.15 ± 0.09
Change in pennation (°)	**GM** [a,b]	7.7 ± 3.4	10.2 ± 2.2 [c]	9.4 ± 3.2	9.7 ± 2.0
	SOL	8.8 ± 2.8	9.8 ± 4.1	9.6 ± 3.1	12.6 ± 3.8
Change in thickness (mm)	**GM**	0.8 ± 0.9	0.6 ± 1.0	0.7 ± 0.7	0.6 ± 0.6
	SOL	3.2 ± 2.9	3.7 ± 2.2	2.5 ± 1.0	3.1 ± 1.4
Force (N)	**AT**	4315 ± 1646	4041 ± 1331	3632 ± 765	3765 ± 917

Values are means ± sd.; [a] $P < 0.05$ interaction effect; [b] $P < 0.05$ main effect of time; [c] $P < 0.05$ comparing pre- and post-intervention test. Architectural gear ratio during stance (AGRs) was calculated as the ratio between muscle length change and fascicle length change during the stance phase.

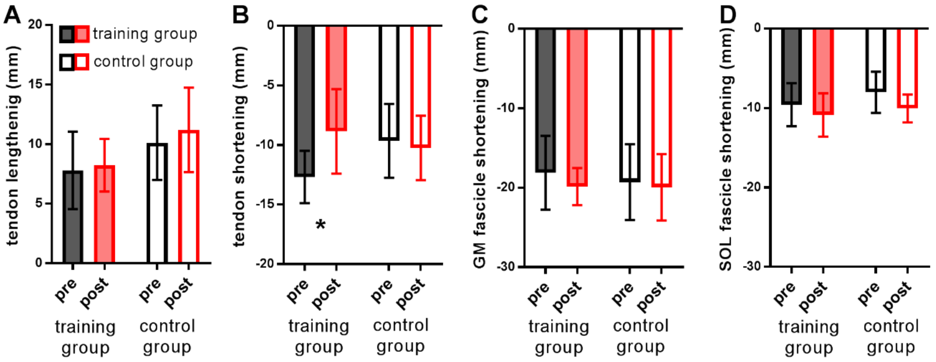


Figure 18. *Achilles tendon shortening and lengthening behaviour (A and B) and gastrocnemius (GM) and soleus (SOL) fascicle shortening behaviour (C and D) during the stance phase of running compared between pre- and post- intervention tests in the training group (filled bars) and the control group (empty bars). Data are means ± s.d.; * P ≤ 0.05 when comparing pre- and post- intervention tests.*

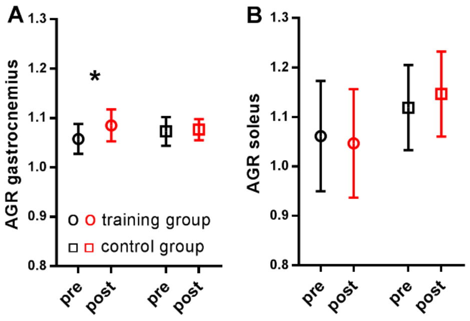


Figure 19. *Architectural gear ratio during the stance phase (AGRs) of running for gastrocnemius medialis (A) and soleus (B) compared between pre- and post- intervention tests in the training group (filled circles) and the control group (empty squares). Data are means ± s.d.; * P ≤ 0.05 when comparing pre and post intervention tests.*

4 Discussion

The purpose of this thesis was to investigate the role of Achilles tendon properties in muscle-tendon interaction when energy is dissipated (during a landing task) and when energy is recycled (during running). An initial step consisted in characterising human muscle-tendon behaviour during running with an unprecedented exhaustive approach and, for the first time, during landing. Subsequently, the aim was to explore the effect of altered external constraints and the effect of a training-induced increase in Achilles tendon stiffness on muscle-tendon interaction during landing and running. Two experimental studies were performed to address these research aims, using a cross-sectional study design and a controlled longitudinal study design.

The cross-sectional study showed that muscle fascicle and tendon behaviour observed during the single-leg landing task supports previous observations on animals, which indicated that elastic structures allow decoupling of changes in fascicle length from that of the MTU. The decoupling enables the buffering role played by the tendon during rapid stretches of the MTU. Contrary to our predictions, fascicle strains did not change when loading was added and instead of the fascicles, elastic elements took up the additional MTU lengthening, preventing higher muscle strains. During running, MTU stretch and shortening behaviour was similar across speed and loading conditions. As expected, different strategies were used to produce the increased positive ankle joint work required when running speed was increased or when load was added. With increasing speed, gastrocnemius and soleus peak muscle activation increased, whereas tendon length changes were unaffected during the stance phase. Contrarily, with loading, Achilles tendon recoil increased, whereas muscle activity was unaltered. Contractile conditions of gastrocnemius and soleus fascicles (with respect to force-length and force-velocity relationship) during stance were preserved across all conditions.

After the ten week training period, Achilles tendon stiffness was increased by 18%. Concomitantly, plantarflexion force, resting muscle thickness and pennation angle increased (by 15, 5 and 5%, respectively). Surprisingly, tendon strain remained similar during energy absorption. Despite no changes in negative work and force during the landing task, gastrocnemius fascicle strain and strain rate were reduced after training. During running, fascicle shortening pattern and velocities were also sustained after the tendon stiffening training intervention. However, increased AGR of gastrocnemius, quantified during the whole stance phase, indicated altered muscle mechanics for this muscle after training. Importantly, Achilles tendon stretch was not altered but tendon recoil was reduced. A simplified overview over how the different conditions affected muscle and tendon behaviour during landing and running is provided in **Table 7**.

Table 7. *Overview of the effects of increased load and speed (running) and of training-induced increased tendon stiffness on fascicle and tendon kinematics and muscle activity during landing and running.*

			Landing		**Running**		
			↑ load	**↑ stiffness**	**↑ load**	**↑ speed**	**↑ stiffness**
Fascicles	**velocity**	**GM**	−	↓	−	−	−
		SOL	−	−	−	−	−
	length change	**GM**	−	↓	−	−	−
		SOL	−	−	−	−	−
Muscle	**activity**	**GM**	−	N/A	−	↑	N/A
		SOL	↑	N/A	−	↑	N/A
Tendon	**stretch**		↑	−	−	−	−
	recoil		−	−	↑	−	↓

GM, gastrocnemius medialis muscle; SOL, soleus muscle; ↑, increase; ↓, decrease; −, no effect, N/A, not applicable.

4.1 Muscle-tendon interaction during a step-landing task

At the onset of negative ankle power, the MTU behaved almost isometrically. However, the individual components of the MTU changed in length. Gastrocnemius and soleus fascicles shortened, while elastic elements and tendon stretched slightly and thus stored energy. Normalised muscle activity, especially of gastrocnemius, was high during this period. The shortening of fascicles after touch-down was consistent with previous reports on animal landings when they were dropped from a rather high height (1.5 m) but not from a low height (0.5 m) (Konow & Roberts, 2015). The apparent connection of higher demands and fascicle shortening in this study may indicate that fascicles would not shorten in humans either with lower demands.

During phase two, the stretch of the MTUs was caused by ankle dorsiflexion and was accompanied by fast increasing joint moments. Tendon and combined elastic elements took up the rapid MTU stretch, storing elastic energy. Simultaneously, muscle fascicles started to actively lengthen and dissipate energy. Importantly, the elongation of elastic tissue reduced fascicle velocities relative to the MTU lengthening velocities. Slower fascicle shortening velocities have been associated with reduced fascicle power input in animals (Konow & Roberts, 2015). If a similar mechanism in humans exists, we may speculate that fascicle power input was lower than that of the MTU in the present task, preventing fibre damage (Roberts & Azizi, 2010). Muscle activity of soleus peaked in the end of this phase, whereas gastrocnemius activity declined. The different activation pattern of the two synergists suggests that they have different roles during the landing despite a similar fascicle length change pattern. Similar to a suggested higher torque contribution of soleus during walking (Cronin et al., 2013), soleus muscle activity may be increased because this mono-articular muscle likely contributes more to the ankle joint moment during the landing task.

During force decay (phase 3), MTU continued to lengthen but at a much slower rate than during force rise in the preceding phase. Contrastingly, elastic elements shortened but tendon alone did not change in length. The combination of MTU lengthening and shortening of elastic elements drove further fascicle lengthening during phase three, indicating that dissipation of energy continued during the end of the landing task. Surprisingly, tendon was still strained by 6% at the end of negative power production at the ankle, possibly caused by the resistance of the body mass.

The discrepancies in elastic elements and Achilles tendon behaviour in phase two and three could possibly result from methodological differences between tendon and elastic element length measurements (Zelik & Franz, 2017). While tendon length was measured directly, assuming a straight line, elastic element length was calculated based on muscle fascicle and MTU length measurements. Alternatively, differences in the mechanical properties of elastic elements such as aponeurosis (as for example shown in Tilp et al., 2012) and other spring-like elements within the muscle (e.g. titin) and the Achilles tendon may have caused these differences.

The buffering function of tendon when energy dissipation is required was first described in animals (Konow et al., 2012). The results of the present study are consistent with these findings and expand on previous investigations focusing on the human triceps surae during tasks where energy was partly dissipated, such as counter movements (Ishikawa et al., 2005; Kurokawa et al., 2001) or stair descent (Spanjaard et al., 2007). Indeed, during drop jumps and stair descent part of the stored elastic energy is arguably used towards push-off work, requiring different fascicle behaviour than during the pure energy dissipation task studied in the present thesis. Timing of muscle activity further distinguished the stair descent from the landing in the present study showing higher gastrocnemius muscle activity during fascicle lengthening. Additionally, we observed that soleus activity was high during the whole energy absorption period.

The uncoupling of fascicle from overall MTU behaviour, enables a delayed and slower fascicle lengthening to dissipate energy. Previous work has linked fascicle strain magnitude and lengthening velocity to muscle damage (Guilhem et al., 2016). Thus, limited fascicle lengthening velocity and likely limited power input to fascicles may provide a protective mechanism against muscle damage during lengthening contractions (Roberts & Konow, 2013).

4.2 The effect of external constraints on muscle-tendon behaviour

In study A, we varied the externally applied constraints during the two different tasks, landing and running.

4.2.1 Increased load during a single-leg step-landing task

Joint and muscle-tendon kinematics were minimally affected by loading during the first phase of the landing. Contrary to our findings, incrementing step height during stair descent caused greater fascicle shortening after touch-down (Spanjaard et al., 2008). The lack of changes in fascicle shortening in the present task may be attributable to differences in energetic requirements for step landing and stair descent. Differences with loading compared to the unloaded condition were indicated by the longer duration of the negative power phase and the increased soleus EMG activity. Larger contribution of the knee joint to compensate for the added loading may also have contributed to preventing greater fascicle strains.

The present expected increase in GRF and joint moment with loading was accompanied by greater ankle dorsiflexion during phase two. Both gastrocnemius and soleus MTUs lengthened more with loading compared to the unloaded condition, whereas increased knee flexion partly offset the effect of increased dorsiflexion on MTU lengthening in the bi-articular gastrocnemius. A greater stretch of tendon and combined elastic elements with loading occurred during phase two, which shows that additional energy was stored in elastic structures and that fascicles were not required to lengthen faster. It may be speculated that preserved fascicle lengthening behaviour between loading conditions prevents strain induced muscle damage, which could be possible due to the increased duration for energy absorption.

Although the MTU stretch was also increased during phase three compared to the unloaded condition, neither lengthening of fascicles nor tendon or elastic elements reflected that change.

Larger variability of ultrasound based measurements compared to joint kinematics may explain this discrepancy (see 4.5 for limitations of ultrasound measurements).

Soleus EMG activity was increased to meet the additional loading. In contrast, gastrocnemius EMG did not increase statistically, which may be again explained by the articularity of the two muscles. Increased co-activation of tibialis anterior, however, probably reflected the higher torque produced by soleus.

In addition to the critical role of elasticity for energy dissipation tasks in general, the increased elastic buffer with loading highlights the important function of elastic tissue in controlling demands during landing tasks. The absorption of additional energy is possible through prolonged duration of the energy absorption phase and presumably the higher force production of soleus.

4.2.2 Increased load and speed during running

The 20% increase in load and in speed were met by different adjustments on muscle-tendon level. Gastrocnemius and soleus fascicle shortening pattern were similar between running speed conditions. In contrast, previous studies examining fascicle operating length when running speed increased had reported a shift towards shorter, presumably unfavourable, operating length in gastrocnemius (Ishikawa & Komi, 2007) and soleus (Lai et al., 2015) for relatively high differences in speed (2 - 6.5 ms^{-1} and 2 - 5 ms^{-1}, respectively). However, for the speeds used in this protocol, the presumably advantageous fascicle operating length - towards the top of the ascending limb - has been maintained. The preservation of fascicle velocities when running speed increases is consistent with previous studies, which did not find an influence of speed on gastrocnemius (Farris & Sawicki, 2012) or soleus (Lai et al., 2015) fascicle shortening velocities. An increase in fascicle velocity may have been expected due to the time constraint imposed by shorter ground contact. This lack of change is deemed beneficial for force production considering the force-velocity relationship of skeletal muscles (Hill, 1938). However, simulations of fascicle behaviour at higher running speeds have indicated an increase in fascicle velocity, suggesting that fascicle velocities are only maintained within certain speed ranges (Dorn et al., 2012).

The present results further show that fascicle behaviour during running did not change with added loading. A similar loading of running guinea fowls (22% of body mass) resulted in an initial fascicle stretch after touch-down (McGowan et al., 2006). This stretch was not observed in human subjects of this study but it may be speculated that the relative demands on the gastrocnemius muscle were higher for the animals, and that a similar stretch could be observed in humans at higher loads.

The difference in change in muscle activity with loading at the two different speed modalities (reduced muscle activity with loading when subjects were running at preferred speed and no change when loading was added at increased speed) may suggest that the effect of loading depends on running speed. Unlike with added load, muscle activity of both muscles was higher when speed increased regardless of loading condition. Previous studies support the finding of increased muscle activity with speed (Kyröläinen et al., 2005; Lai et al., 2015). Muscle activity and fascicle behaviour were adjusted in similar ways in gastrocnemius and soleus across speed and loading conditions despite the generally different roles of the two muscles during running (Lai et al., 2018). The differences between plantar flexor behaviour could, however, indicate different adjustments in muscle activity of these muscles for changes larger than 20%.

A previous simulation study predicted that stepwise increases in running speed from 2.1 to 9 ms^{-1} would increase the elastic contribution to positive MTU work of soleus, whereas the contribution of gastrocnemius elastic strain energy remained unchanged up to 7 ms^{-1} (Lai et al., 2014). The present results agree with these predictions by showing similar Achilles tendon strain and recoil using *in vivo* measurements of the Achilles tendon at speeds below 4 ms^{-1}, suggesting a constant elastic energy contribution within the studied speed range.

Loading the subjects, unlike the increase in speed, lead to an increased tendon recoil amplitude and velocity, which was consistent at both speed modalities. The greater tendon recoil suggests a larger energy return with loading. Despite this evidence for increased elastic energy utilisation with loading, tendon stretch after touch-down did not increase statistically. Albeit speculative, changes in muscle and tendon deformation may explain this discrepancy. A greater proportion of energy may have been stored transversally in the gastrocnemius aponeurosis, which has been shown to reduce longitudinal strain during isometric contractions (Farris et al., 2013) and increases with contraction force due to radial expansion of muscle fascicles (Arellano et al., 2016; Azizi & Roberts, 2009). The additional elastic energy stored transversally may present a mechanism to increase positive ankle joint work during the push-off phase without altering joint kinematics and coordination. The discrepancy of unchanged tendon stretch and increased tendon recoil could result from the longer time on the ground that is available when running loaded, which may have enabled the increased tendon recoil.

In summary, time constrains and the availability of energy imposed by increased speed and loading likely modulate muscle-tendon interaction during running to meet the increased requirements for positive ankle joint work. While elastic energy utilisation was favoured with loading, a higher muscle activation was required when speed increased. Interestingly, the present data further suggest a prioritisation to conserve contractile conditions during running since fascicle shortening pattern of gastrocnemius and soleus were preserved across conditions despite differences in MTU velocities, ground impulse and estimated tendon forces. The consistency of adjustments with speed and loading across modalities suggest that the mechanisms to increase load and speed may be independent from each other within the tested speed and loading range.

4.2.3 Summary altered external constraints

Our data showed that additional loading is modulated by higher tendon strain, which seems during movements requiring energy dissipation and energy conservation. More elastic energy may be stored during landing and recycled during running, whereas contractile conditions for fascicles can be preserved. Increased speed restricted the available time to produce forces on the ground during running and we showed that higher muscle activity was required with this experimental constraint. It may be speculated that a similar reduction in time to absorb energy during landing would also require higher muscle activity.

4.3 The effect of increased tendon stiffness on muscle-tendon behaviour

Unlike other independent variables (i.e. running speed, load), the increase in Achilles tendon stiffness induced by training in study B could not be induced precisely and inherently varied between subjects. The mean increase in tendon stiffness of 18% induced by the present protocol is however in agreement with previous studies where training was based on isometric contractions (Albracht & Arampatzis, 2013; Arampatzis et al., 2010). Despite this increase in tendon stiffness, strain was surprisingly not reduced significantly during the isometric ramp contractions. Yet, this discrepancy may be due to methodological shortcomings during strain measurements (Seynnes et al., 2015) and has also been reported by other studies (e.g. Arampatzis et al., 2007). A mean coefficient of variation of 15% for strain measurements in the control group in this study illustrates the relatively poor sensitivity. The choice of training modalities (i.e. isometric contractions and high rate of force development) was chosen to limit muscle architecture adaptations. However, the type of training may have prevented greater increases in tendon stiffness.

The increase in tendon stiffness was accompanied by increases in gastrocnemius resting pennation angle and thickness (by 5% each). The relation between these variables and the number of sarcomeres in parallel is consistent with the increase in plantarflexion strength (15%). Importantly, fascicle length did not change, suggesting that the effects of changes in series elasticity on muscle-tendon interaction were not mitigated by an increase in sarcomeres in-series (Lichtwark et al., 2018).

4.3.1 Increased tendon stiffness and single-leg landing task

The execution of the landing task was overall similar when comparing joint angle excursion and MTU lengthening between baseline- and post-training intervention tests. However, the training group landed in a more dorsiflexed position (i.e. ankle angle at touch-down) and a similar trend was observed in the controls despite the practice trials that were included in the protocol. This change in landing strategy may be attributable to improved task execution and thus insufficient familiarization or to effects of the training intervention that were not accounted for, such as changes in rate of force development or optimal angle of torque production.

Despite unchanged joint moments, and against the hypothesis, tendon strain was not reduced after training. Similar to the unaltered tendon strain during isometric contractions (see chapter 4.3), it can be speculated that the increase in stiffness in the present study was not sufficient to reduce longitudinal tendon strain during the landing task or that methodological limitations prevented the detection of changes in strain. Alternatively, the multidirectional nature of strains in the proximal part of the Achilles tendon (Farris et al., 2013), support the existence of direction specific strain changes after training that affect longitudinal tendon strain. Furthermore, the small changes in muscle architecture or the contribution of different plantar flexor muscles may have affected the longitudinal strain of the tendon.

Although longitudinal tendon strain was similar, a reduction in active lengthening of gastrocnemius during the landing was observed after training. The reduction in fascicle lengthening was accompanied by a lower fascicle velocity in this muscle. Lower lengthening velocities are associated with a reduction in muscle fibre damage caused by fast eccentric contractions (Proske & Morgan, 2001). A similar trend of reduced lengthening amplitude and velocity was observed in the soleus but, unlike for the gastrocnemius, it disappeared when accounting for MTU length changes. These results suggest different roles of the triceps surae muscles during landing and/or a smaller effect of training on the soleus.

The reduction in gastrocnemius fascicle lengthening should also be considered in relation to the remarkable offset in fascicle length at touch-down seen after training. A possible reason for the 8% longer fascicle length could be the stiffening of elastic tissue, such as aponeurosis or proximal tendon, which would constrain fascicles to longer lengths at touch-down. This may also explain why there was no offset observed for soleus fascicles. Despite differences in ankle joint angle at touch-down, the normalization of fascicle length to MTU length confirmed that fascicle length offset was not solely due to the difference the differences in task execution. This offset, resulting in longer fascicle length, may have increased the contraction force, due to the force-length relationship of this muscle (operating at the ascending limb), possibly allowing to dissipate a similar amount of energy despite less lengthening and perhaps enabling a better control at touch-down. However, the changes in forces and hence energy storage and dissipation are difficult to interpret with the present data. Higher tendon stiffness and plantarflexion force after training would affect energy storage but the calculation of tendon force and also joint work does not indicate a pre-post difference in kinetic measurements challenging this interpretation. The unchanged forces suggest that a similar amount of energy may have been dissipated despite the reduction in fascicle lengthening.

In summary, the training-induced increase in Achilles tendon stiffness did not limit the buffering function of the tendon during energy dissipation. The unchanged tendon strain could not directly be linked to the surprising decrease in gastrocnemius fascicle lengthening and velocity, which was observed despite similar forces before and after training. Altered transverse aponeurosis strain may contribute to explaining these findings but the mechanisms for altered fascicle behaviour and changes in energy flow remain unclear.

4.3.2 Increased tendon stiffness and running

Against the main hypothesis, we did not observe an influence of increased Achilles tendon stiffness on tendon elongation during running. According to the ratio of similar tendon force, which was estimated from inverse dynamics, to the 18% greater stiffness, tendon elongation should have been reduced by about 19% and it is not clear why such a difference was not observed. The unaltered tendon strain is consistent with a previous study reporting no changes in series elastic element strain during running after a training intervention designed to stiffen the Achilles tendon (Albracht & Arampatzis, 2013). The authors proposed that the unaltered elastic stretch, despite higher tendon stiffness, would have been caused by increased forces and joint moments, which the present study could not confirm since joint kinetics were similar after training. The same methodological limitations as referred to in previous chapter, which will be discussed in more depth later (chapter 4.5), may have affected the sensitivity of tendon strain measurements. Likewise, the lack of change in longitudinal tendon strain as predicted may result from altered deformation pattern of the aponeurosis so that the longitudinal tendon stretch remained consistent because of a reduction in transversal deformation.

As predicted and in contrast to the elongation, Achilles tendon recoil during the push-off phase was reduced after training. However, the unchanged joint moments and kinematics measured after training suggest that elastic energy return may have been similar. A reduced tendon recoil would suggest that energy return during late-stance was decreased. However, the higher stiffness, allowing to store more energy for a given elongation, may partly have compensated for this effect. Thus, Achilles tendon strain patterns and hence the way energy was stored were altered during running stance, presumably via the increase in tendon stiffness.

In line with unaltered tendon elongation patterns, gastrocnemius and soleus fascicle length and velocity during the stance phase were not different after training. Although a previous study also found no differences in gastrocnemius shortening behaviour following a similar training intervention (Albracht & Arampatzis, 2013), these results were unexpected considering the typical in series muscle-tendon models. Despite unchanged fascicle length, an increase in gastrocnemius pennation angle change during the stance phase showed a difference in the contractile behaviour of the gastrocnemius muscle despite the unchanged fascicle shortening pattern. The increase in AGRs was complementary to the change in pennation angle, although gearing was only estimated for the whole stance phase and not instantaneously. These changes in contractile pattern should be interpreted with caution because the mechanistic links between muscle gearing, force level (Azizi et al., 2008; Dick & Wakeling, 2017) and aponeurosis stiffness (Eng & Roberts, 2018) have thus far only been established in cross-sectional, well controlled comparisons. In the present study, the changes in AGRs could simply be the geometrical consequence of the increase in resting pennation angle and muscle thickness after training. In contrast, they could be connected to radial aponeuroses stiffness, which has likely been affected by the training intervention and is of particular importance at high forces (Eng & Roberts, 2018). This in vitro protocol also showed the influence of radial aponeurosis stiffness on muscle gearing. Further it has been established that an increase in muscle thickness is associated with increased gearing, whereas AGR is not affected by an increase in muscle width (Eng et al., 2018). Admittedly, the increased muscle expansion in thickness with higher gearing was not observed in this study. However, muscle thickness measurements may have a limited validity here, due to the compression created by the tight strapping of the ultrasound transducer and certainly because thickness was only estimated using simple trigonometry based on fascicle length and pennation angle.

Despite the hypothesised higher susceptibility of the soleus to changes in tendon stiffness, fascicle shortening pattern and velocity in this muscle remained unchanged after training, similarly to the gastrocnemius. A relatively insufficient increase in free tendon stiffness to alter the elongation of this region could possibly explain the unchanged soleus fascicle behaviour appreciably (see chapter 4.5 for the discussion on measurements of the free tendon). Unaltered AGRs of soleus suggests that, in contrast to gastrocnemius, transverse loading patterns did not result in significant differences in muscle expansion in thickness after training, and that the two muscles were affected differently by the training intervention. Differences in contractile behaviour may be attributed to the anatomical organisation of the muscles.

These data showed that a training-induced increase in tendon stiffness directly affected Achilles tendon recoil and gastrocnemius – but not soleus - muscle mechanics during running. Changes in gastrocnemius muscle mechanics were illustrated by the increased change in pennation angle and the increased gear ratio during stance and may be connected to altered modulation of aponeurosis stiffness after training. A modulation of aponeurosis stiffness may also have allowed the preservation of gastrocnemius and soleus fascicle shortening pattern and altered the way elastic energy was stored in the tendon after training.

4.3.3 Summary altered Achilles tendon stiffness

The differences in adjustments in muscle fascicle behaviour during landing and running following a training-induced increase in Achilles tendon stiffness may indicate that adjustments are task dependent. However, similar mechanisms may have resulted in the unchanged tendon stretch observed during both tasks, which may be explained by altered transverse aponeurosis strains that affect the measured longitudinal tendon strain. Also consistent across tasks were the discrepancies between the predicted adjustments of muscle and tendon behaviour based on structures acting in series and the findings in both tasks. The preserved contraction behaviour in soleus was another similarity between landing and running after training.

4.4 Between studies comparison of fascicle and tendon behaviour

The results from this thesis showed that fascicle length and velocity were preserved under most examined conditions during landing and running (except for the landing task after training). It is important to note that the present findings apply to the limited range of increased speed, loading and stiffness used in this thesis (20%, 20% and 18%, respectively) and remain to be verified for larger ranges. However, the remarkably consistent fascicle behaviour within the tested ranges suggests a prioritisation of other strategies within the MTU than modifying fascicle length and velocity, possibly to sustain beneficial contractile conditions for the muscles with respect to force-length and force-velocity relationship of the muscles.

Differences in fascicle behaviour were reported previously between gastrocnemius and soleus during walking (Cronin et al., 2013) and running (Lai et al., 2018). We showed in our studies that the two muscles may also adjust differently under different task configurations or after a training-induced tendon stiffening. The training-induced increase in stiffness resulted in an offset of gastrocnemius fascicle length during landing and gastrocnemius contraction behaviour was altered during running after training, whereas no significant differences were seen in the soleus fascicle behaviour. Thus, the present findings suggest a higher susceptibility of gastrocnemius to adjust to varying conditions. While soleus may be the main contributor to forward propulsion (Hamner et al., 2010), gastrocnemius has a multi-functional role and additionally transfers mechanical energy between ankle and knee joint (Kaya et al., 2005). Hence it is speculated that the transfer of joint work between ankle and knee may have changed after training due to the modulation of the bi-articular gastrocnemius muscle.

A reoccurring observation for Achilles tendon behaviour in this thesis was a lack in changes of longitudinal strain when it was expected based on in-series muscle-tendon models as discussed in the specific sections (4.3 - 4.4). This phenomenon appeared especially for tendon elongation rather than tendon recoil. The repeated occurrence across the different tasks and study designs suggests that similar factors may be responsible despite the contrasting conditions. Based on recent literature (as summarised in the review by Raiteri, 2018) it was speculated that the mechanisms for asymmetrical adjustments of tendon behaviour and for the lack of predicted adjustments are connected to variable aponeurosis stiffness, however this hypothesis remains to be confirmed.

4.5 Methodological considerations

The methodology of the present thesis contains some limitations and necessary assumptions, which may have affected the results and should be addressed in greater detail here. One limitation was that the mechanical properties and strain patterns of the free tendon and the proximal tendon part were not examined separately due to technical difficulties although the literature indicates differences in strain between the two parts. A recent study showed for example that higher longitudinal strain occurs in the soleus (i.e. deep) part of the Achilles tendon than in the gastrocnemius (i.e. superficial) part during isometric contractions (Clark & Franz, 2018). During running the strain of so-called series elastic elements of the soleus was also higher than that in the gastrocnemius (Lai et al., 2018). On the basis of these studies, we assumed higher sensitivity of soleus to changes in tendon stiffness. A possible reason for that no significant changes in soleus were observed may be that free tendon strain was not sufficiently affected but future studies are required to devise methodologies to quantify this parameter during locomotion.

The lack of reduction in tendon strain (during landing and running after training and during running with loading) was ascribed to changes in contractile patterns but could also reflect methodological shortcomings. The measurement of tendon length used in this thesis has been shown to better reflect elastic energy storage and return than when estimating length changes in all combined elastic elements (Zelik & Franz, 2017). Nevertheless, several factors may still limit this approach. For example, as noted in the context of isometric contractions, the determination of tendon slack length is currently difficult to obtain because collagen crimp patterns cannot be determined *in vivo* (Seynnes et al., 2015). Our comparison of tendon strain at standardised time points (i.e. in a similar joint configuration) in the movement would not account for possible changes in tendon slack length after the training intervention. Furthermore, Achilles tendon was modelled as straight line, which may have led to an underestimation of tendon length (Obst et al., 2017; Stosic & Finni, 2011). The underestimation of tendon length could further result in an underestimation in tendon length change in the present data. In turn, tendon length changes might have been overestimated when considering that Achilles tendon is more curved when the ankle joint is in a plantar flexed position than in a dorsiflexed position.

In chapters 4.1-4.3, discrepancies between tendon strain measurements and predictions based on in-series models or between stretch and recoil magnitudes, were attributed to the complexity of relative contributions of elastic tissues. Study A demonstrated differences between tendon and elastic element behaviour during landing, which have previously been shown to occur during running (Lichtwark & Wilson, 2006). The discrepancies in elastic tissue behaviour highlight the importance to measure contributions of different elastic tissues such as aponeurosis whose distinct role received more attention recently (Raiteri, 2018). In addition, the body of literature lacks longitudinal investigations on the response of aponeuroses mechanical properties to chronic loading and its effects on muscle-tendon behaviour during locomotion. This gap of knowledge limits the interpretation of the results from study B and the speculations made about dynamic muscle shape changes.

A simplified version of the originally proposed approach by Brainerd and Azizi (2005) to calculate AGR was used in Study B to quantify the relationship of muscle and fascicle length changes during the stance phase of running. To reduce the influence of the noise from differentiating the relatively variable fascicle length data from ultrasound, we calculated the gear ratio using the strain of muscle and fascicles over the whole stance phase and not the velocities. However, since fascicles shorten throughout the stance phase and the stance phase had the same duration after training, this value provides meaningful information about the relation between muscle and fascicle shortening during stance.

A more frequently discussed limitation of the methods used in the present thesis is the use of ultrasonography to image muscle fascicles during locomotion (for reviews see Cronin & Lichtwark, 2013; Kwah et al., 2013). Fascicle tracking has been found to have good reliability (Cronin et al., 2011; Gillett et al., 2013). However, the fascicle segmentation in the initial frame is more variable than in the rest of the sequence, where information from preceding frames inform the analysis (Aeles et al., 2017). This limitation is of particular interest for the findings from Study B, indicating that fascicle length of gastrocnemius was offset at touch-down. Precautions were therefore taken to minimise the risk of error for this variable. All data sets were analysed by the same investigator, except for the step landing in study A where the large number of subjects was divided between two investigators. In addition, the inclusion of a control group in study B demonstrated the reliability of fascicle length measurements and all other variables.

Although the estimation of tendon forces seems reliable (with a CV of 4% between testing sessions in the control group during running), a question about its validity remains since the estimation used here is based on several assumptions. For example, the estimation of Achilles tendon moment arm, which is especially challenging when large changes in joint angles occur, may have influenced the magnitude of forces. Furthermore, treating the foot as a single rigid body when estimating ankle moment, as in the model used in this thesis, can overestimate muscle-tendon power generated around the ankle (Zelik & Honert, 2018). An overestimation of ankle joint moment and hence overestimation of tendon forces would however be less important for our study due to the repeated-measures design. In addition, the contribution of different plantar flexor muscles may vary during stance and/or after training.

5 Conclusion and perspectives

5.1 Concluding remarks

This thesis expands the current understanding of the modulation of muscle-tendon behaviour during landing tasks and running using *in vivo* measurements in humans. The following conclusions were drawn:

1. As suggested by animal studies, the human Achilles tendon buffers the rapid MTU stretch during landing, preventing greater fascicle strains. During this type of task, the tendon stretch is commensurate with external loading constraints to take up additional energy and fascicle strains were not affected by loading.
2. Increased tendon stiffness does not reduce tendon elongation during landing under similar force conditions. The discrepant reduction in gastrocnemius fascicle strains and unchanged longitudinal tendon strain remain unexplained.
3. Increased running speed and increased loading during running result in distinct muscle-tendon interactions. At higher speed, increased positive work is achieved with increased muscle activity. In contrast, elastic energy utilisation is prioritised with increased loading. Despite differences in kinetics across conditions, contractile conditions of gastrocnemius and soleus are preserved when either of the external constraints (i.e. loading and speed) are imposed.
4. Increased tendon stiffness reduces tendon recoil and likely alters the way energy is stored and released during running. Increased gastrocnemius AGRs indicates altered contraction conditions for this muscle, while gastrocnemius and soleus fascicle operating length and velocity remain unchanged.

5.2 Future directions

The results of this thesis raise a number of questions that should be addressed in further research. While we have shown how muscle-tendon behaviour adjusts to selected experimental changes, the present findings have to be verified for higher increases in speed, load and tendon stiffness to further improve our understanding of muscle-tendon modulation. More contrasting conditions may increase the differences in variables and/or induce other mechanisms. The differences in muscle-tendon interaction in the different experimental conditions in this thesis clearly show that different structures on muscle-tendon level adjustment and this diversity may indicate that more *in vivo* studies are required that investigate other conditions to further improve our understanding of MTU function. Possible experimental conditions would be changes in other external constraints such as cadence, shoe cushioning or stiffness of the underground and changes in other mechanical properties of the MTU would be interesting to investigate. In addition, the partly unexpected findings of the present thesis indicate that examining the modulation of muscle-tendon behaviour during tasks where power is amplified, such as squat jumps, would further improve our understanding of MTU function.

Future studies examining the connection between external constraints or tendon mechanical properties and muscle-tendon function should include transverse tendon and aponeurosis strain measurements to verify the hypotheses proposed in this work. A first step could be to explore how muscle AGR and longitudinal aponeurosis stiffness are affected by training-induced changes in tendon stiffness and/or plantarflexion force in better controlled conditions than in this thesis (e.g. in a dynamometer) to improve our understanding of how muscle gearing is affected by training. The incorporation of motion capture and B-mode ultrasonography, as used in this thesis to estimate tendon length, could be improved to assess muscle and tendon behaviour in several dimensions and/or different regions. Using three-dimensional ultrasonography in a longitudinal study design to assess aponeurosis shape changes during isometric contractions (as for example in Farris et al. (2013) or Raiteri et al. (2016)) would be a possible approach. Ultimately, the possibility to obtain three-dimensional ultrasound images with high acquisition frequencies would be an improvement in technology opening many possibilities to study multidirectional muscle-tendon behaviour during locomotion.

Considering the remarkable results for Achilles tendon strain and the differences in gastrocnemius and soleus contraction behaviour, it would also be an interesting prospect to examine not only the gastrocnemius tendon but also the free tendon stiffness after a similar resistance training protocol and whether free tendon strain differs from that of the gastrocnemius tendon during different tasks.

Overall, loading and speed constraints and altered stiffness did not systematically result in the predicted adjustments - in either muscle or tendon – when studied using simple in-series muscle-tendon models. This highlights the importance of further *in vivo* studies to improve our understanding of muscle-tendon interaction during locomotion and the necessity to use refined muscle-tendon models.

6 References

Aagaard, P., Andersen, J. L., Dyhre-Poulsen, P., Leffers, A.-M., Wagner, A., Magnusson, S. P., . . . Simonsen, E. B. (2001). A mechanism for increased contractile strength of human pennate muscle in response to strength training: changes in muscle architecture. *J. Physiol., 534*(2), 613-623.

Aeles, J., Lichtwark, G. A., Lenchant, S., Vanlommel, L., Delabastita, T., & Vanwanseele, B. (2017). Information from dynamic length changes improves reliability of static ultrasound fascicle length measurements. *PeerJ, 5*, e4164.

Aerts, P. (1998). Vertical jumping in Galago senegalensis: the quest for an obligate mechanical power amplifier. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 353*(1375), 1607-1620.

Albracht, K., & Arampatzis, A. (2013). Exercise-induced changes in triceps surae tendon stiffness and muscle strength affect running economy in humans. *Eur. J. Appl. Physiol., 113*(6), 1605-1615.

Alegre, L. M., Jiménez, F., Gonzalo-Orden, J. M., Martín-Acero, R., & Aguado, X. (2006). Effects of dynamic resistance training on fascicle lengthand isometric strength. *J. Sports Sci., 24*(5), 501-508.

Alexander, R. M., & Bennet-Clark, H. C. (1977). Storage of elastic strain energy in muscle and other tissues. *Nature, 265*(5590), 114-117.

Alexander, R. M., & Vernon, A. (1975). The mechanics of hopping by kangaroos (Macropodidae). *J. Zool., 177*(2), 265-303.

Arampatzis, A., De Monte, G., Karamanidis, K., Morey-Klapsing, G., Stafilidis, S., & Brüggemann, G.-P. (2006). Influence of the muscle-tendon unit's mechanical and morphological properties on running economy. *J. Exp. Biol., 209*(17), 3345-3357.

Arampatzis, A., Karamanidis, K., & Albracht, K. (2007). Adaptational responses of the human Achilles tendon by modulation of the applied cyclic strain magnitude. *J. Exp. Biol., 210*(15), 2743-2753.

Arampatzis, A., Morey-Klapsing, G., Karamanidis, K., DeMonte, G., Stafilidis, S., & Brüggemann, G.-P. (2005). Differences between measured and resultant joint moments during isometric contractions at the ankle joint. *J. Biomech., 38*(4), 885-892.

Arampatzis, A., Peper, A., Bierbaum, S., & Albracht, K. (2010). Plasticity of human Achilles tendon mechanical and morphological properties in response to cyclic strain. *J. Biomech., 43*(16), 3073-3079.

Arellano, C. J., Gidmark, N. J., Konow, N., Azizi, E., & Roberts, T. J. (2016). Determinants of aponeurosis shape change during muscle contraction. *J. Biomech., 49*(9), 1812-1817.

Arnold, E. M., Hamner, S. R., Seth, A., Millard, M., & Delp, S. L. (2013). How muscle fiber lengths and velocities affect muscle force generation as humans walk and run at different speeds. *J. Exp. Biol., 216*(11), 2150-2160.

Astley, H. C., & Roberts, T. J. (2012). Evidence for a vertebrate catapult: elastic energy storage in the plantaris tendon during frog jumping. *Biol. Lett., 8*(3), 386-389.

Azizi, E., Brainerd, E. L., & Roberts, T. J. (2008). Variable gearing in pennate muscles. *PNAS, 105*(5), 1745-1750.

Azizi, E., Halenda, G. M., & Roberts, T. J. (2009). Mechanical properties of the gastrocnemius aponeurosis in wild turkeys. *Integr. Comp. Biol., 49*(1), 51-58.

Azizi, E., & Roberts, T. J. (2009). Biaxial strain and variable stiffness in aponeuroses. *J. Physiol., 587*(17), 4309-4318.

Baroni, B. M., Geremia, J. M., Rodrigues, R., Azevedo, F. R., Karamanidis, K., & Vaz Marco, A. (2013). Muscle architecture adaptations to knee extensor eccentric training: Rectus femoris vs. vastus lateralis. *Muscle Nerve, 48*(4), 498-506.

Bell, A. L., Brand, R. A., & Pedersen, D. R. (1989). Prediction of hip joint centre location from external landmarks. *Hum. Mov. Sci., 8*(1), 3-16.

Bennett, M. B., Ker, R. F., Imery, N. J., & Alexander, R. (1986). Mechanical properties of various mammalian tendons. *J. Zool., 209*(4), 537-548.

Biewener, A. A., Konieczynski, D. F., & Baudinette, R. V. (1998). In vivo muscle force-length behavior during steady-speed hopping in tammar wallabies. *Journal of Experimantal Biology*(0022-0949 (Print)), 1681-1694.

Blazevich, A. J., Cannavan, D., Coleman, D. R., & Horne, S. (2007). Influence of concentric and eccentric resistance training on architectural adaptation in human quadriceps muscles. *J. Appl. Physiol., 103*(5), 1565.

Blazevich, A. J., Gill, N. D., & Zhou, S. (2006). Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. *J. Anat., 209*(3), 289-310.

Blazevich, A. J., & Giorgi, A. (2001). Effect of testosterone administration and weight training on muscle architecture. *Med. Sci. Sports Exerc., 33*(10), 1688-1693.

Bobbert, M. (2001). Dependence of human squat jump performance on the series elastic compliance of the triceps surae: a simulation study. *J. Exp. Biol., 204*(3), 533-542.

Bojsen-Møller, J., Magnusson, S. P., Rasmussen, L. R., Kjaer, M., & Aagaard, P. (2005). Muscle performance during maximal isometric and dynamic contractions is influenced by the stiffness of the tendinous structures. *J. Appl. Physiol., 99*(3), 986-994.

Brainerd, E. L., & Azizi, E. (2005). Muscle fiber angle, segment bulging and architectural gear ratio in segmented musculature. *J. Exp. Biol., 208*(17), 3249.

Burgess, K. E., Connick, M. J., Graham-Smith, P., & Pearson, S. J. (2007). Plyometric vs. Isometric Training influences on Tendon Properties and Muscle Output. *J. Strength Cond. Res., 21*(3).

Burkholder, T. J., Fingado, B., Baron, S., & Lieber Richard, L. (1994). Relationship between muscle fiber types and sizes and muscle architectural properties in the mouse hindlimb. *J. Morphol., 221*(2), 177-190.

Butler, D. L., Grood, E. S., Noyes, F. R., & Zernicke, R. E. (1978). Biomechanics of ligaments and tendons. *Exerc. Sport Sci. Rev., 6*(1).

Cavagna, G. A., Saibene, F. P., & Margaria, R. (1964). Mechanical work in running. *J. Appl. Physiol., 19*(2), 249-256.

Clark, W. H., & Franz, J. R. (2018). Do triceps surae muscle dynamics govern non-uniform Achilles tendon deformations? *PeerJ, 6*, e5182.

Couppé, C., Kongsgaard, M., Aagaard, P., Vinther, A., Boesen, M., Kjaer, M., & Magnusson, S. P. (2013). Differences in tendon properties in elite badminton players with or without patellar tendinopathy. *Scand. J. Med. Sci. Sports, 23*(2), e89-e95.

Cronin, N. J., Avela, J., Finni, T., & Peltonen, J. (2013). Differences in contractile behaviour between the soleus and medial gastrocnemius muscles during human walking. *J. Exp. Biol., 216*(5), 909-914.

Cronin, N. J., Carty, C. P., Barrett, R. S., & Lichtwark, G. (2011). Automatic tracking of medial gastrocnemius fascicle length during human locomotion. *J. Appl. Physiol., 111*(5), 1491-1496.

Cronin, N. J., & Lichtwark, G. (2013). The use of ultrasound to study muscle-tendon function in human posture and locomotion. *Gait Posture, 37*(3), 305-312.

Dick, T. J. M., Arnold, A. S., & Wakeling, J. M. (2016). Quantifying Achilles tendon force in vivo from ultrasound images. *J. Biomech., 49*(14), 3200-3207.

Dick, T. J. M., & Wakeling, J. M. (2017). Shifting gears: dynamic muscle shape changes and force-velocity behaviour in the medial gastrocnemius. *J. Appl. Physiol., 123*(6), 1433-1442.

Dorn, T. W., Schache, A. G., & Pandy, M. G. (2012). Muscular strategy shift in human running: dependence of running speed on hip and ankle muscle performance. *J. Exp. Biol., 215*(11), 1944-1956.

Duclay, J., Martin, A., Duclay, A., Cometti, G., & Pousson, M. (2009). Behavior of fascicles and the myotendinous junction of human medial gastrocnemius following eccentric strength training. *Muscle Nerve, 39*(6), 819-827.

Edman, K. A., Elzinga, G., & Noble, M. I. (1978). Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *J. Physiol., 281*(1), 139-155.

Eng, C. M., Azizi, E., & Roberts, T. J. (2018). Structural determinants of muscle gearing during dynamic contractions. *Integr. Comp. Biol.*, icy054-icy054.

Eng, C. M., & Roberts, T. J. (2018). Aponeurosis influences the relationship between muscle gearing and force. *J. Appl. Physiol.*

Ettema, G. J. C., & Huijing, P. A. (1989). Properties of the tendinous structures and series elastic component of EDL muscle-tendon complex of the rat. *J. Biomech.*, *22*(11), 1209-1215.

Farley, C. T., Blickhan, R., Saito, J., & Taylor, C. R. (1991). Hopping frequency in humans: a test of how springs set stride frequency in bouncing gaits. *J. Appl. Physiol.*, *71*(6), 2127-2132.

Farris, D. J., & Lichtwark, G. A. (2016). UltraTrack: Software for semi-automated tracking of muscle fascicles in sequences of B-mode ultrasound images. *Comput. Methods Programs Biomed.*, *128*, 111-118.

Farris, D. J., Lichtwark, G. A., Brown, N. A. T., & Cresswell, A. G. (2016). The role of human ankle plantar flexor muscle–tendon interaction and architecture in maximal vertical jumping examined in vivo. *J. Exp. Biol.*, *219*(4), 528.

Farris, D. J., & Sawicki, G. S. (2012). Human medial gastrocnemius force–velocity behavior shifts with locomotion speed and gait. *PNAS*, *109*(3), 977-982.

Farris, D. J., Trewartha, G., McGuigan, M. P., & Lichtwark, G. A. (2013). Differential strain patterns of the human Achilles tendon determined in vivo with freehand three-dimensional ultrasound imaging. *J. Exp. Biol.*, *216*(4), 594-600.

Farris, D. J., Trewartha, G., & Polly McGuigan, M. (2011). Could intra-tendinous hyperthermia during running explain chronic injury of the human Achilles tendon? *J. Biomech.*, *44*(5), 822-826.

Fenn, W. O. (1924). The relation between the work performed and the energy liberated in muscular contraction. *J. Physiol.*, *58*(6), 373-395.

Finni, T., Peltonen, J., Stenroth, L., & Cronin, N. J. (2013). Viewpoint: On the hysteresis in the human Achilles tendon. *J. Appl. Physiol.*, *114*(4), 515-517.

Fletcher, J. R., Esau, S. P., & MacIntosh, B. R. (2010). Changes in tendon stiffness and running economy in highly trained distance runners. *Eur. J. Appl. Physiol.*, *110*(5), 1037-1046.

Fouré, A., Nordez, A., & Cornu, C. (2010). Plyometric training effects on Achilles tendon stiffness and dissipative properties. *J. Appl. Physiol.*, *109*(3), 849-854.

Fouré, A., Nordez, A., & Cornu, C. (2013). Effects of eccentric training on mechanical properties of the plantar flexor muscle-tendon complex. *J. Appl. Physiol.*, *114*(5), 523-537.

Franchi, M. V., Atherton, P. J., Reeves, N. D., Flück, M., Williams, J., Mitchell, W. K., . . . Narici, M. V. (2014). Architectural, functional and molecular responses to concentric and eccentric loading in human skeletal muscle. *Acta Physiol., 210*(3), 642-654.

Fukashiro, S., Rob, M., Ichinose, Y., Kawakami, Y., & Fukunaga, T. (1995). Ultrasonography gives directly but noninvasively elastic characteristic of human tendon in vivo. *Eur. J. Appl. Physiol. Occup. Physiol., 71*(6), 555-557.

Fukunaga, T., Kubo, K., Kawakami, Y., Fukashiro, S., Kanehisa, H., & Maganaris, C. N. (2001). In vivo behaviour of human muscle tendon during walking. *Proc. R. Soc. Lond. B. Biol. Sci., 268*(1464), 229-233.

Gabaldón, A. M., Nelson, F. E., & Roberts, T. J. (2008). Relative shortening velocity in locomotor muscles: turkey ankle extensors operate at low V/Vmax. *Am. J. Physiol. Regul. Integr. Comp. Physiol., 294*(1), R200.

Gans, C. (1982). Fibre architecture and muscle function. *Exerc. Sport Sci. Rev., 10*(1).

Gelse, K., Pöschl, E., & Aigner, T. (2003). Collagens—structure, function, and biosynthesis. *Adv. Drug Deliv. Rev., 55*(12), 1531-1546.

Geremia, J. M., Baroni, B. M., Bobbert, M. F., Bini, R. R., Lanferdini, F. J., & Vaz, M. A. (2018). Effects of high loading by eccentric triceps surae training on Achilles tendon properties in humans. *Eur. J. Appl. Physiol., 1-12*.

Gillett, J. G., Barrett, R. S., & Lichtwark, G. A. (2013). Reliability and accuracy of an automated tracking algorithm to measure controlled passive and active muscle fascicle length changes from ultrasound. *Comput. Methods Biomech. Biomed. Engin., 16*(6), 678-687.

Goldspink, G. (1985). Malleability of the motor system: a comparative approach. *J. Exp. Biol., 115*(1), 375.

Gordon, A. M., Huxley, A. F., & Julian, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol., 184*(1), 170-192.

Griffiths, R. I. (1991). Shortening of muscle fibres during stretch of the active cat medial gastrocnemius muscle: the role of tendon compliance. *J. Physiol., 436*, 219-236.

Guex, K., Degache, F., Morisod, C., Sailly, M., & Millet, G. P. (2016). Hamstring Architectural and Functional Adaptations Following Long vs. Short Muscle Length Eccentric Training. *Front. Physiol., 7*, 340.

Guilhem, G., Cornu, C., Maffiuletti, N. A., & Guével, A. (2013). Neuromuscular adaptations to isoload versus isokinetic eccentric resistance training. *Med. Sci. Sports Exerc., 45*(2), 326-335.

Guilhem, G., Doguet, V., Hauraix, H., Lacourpaille, L., Jubeau, M., Nordez, A., & Dorel, S. (2016). Muscle force loss and soreness subsequent to maximal eccentric contractions depend on the amount of fascicle strain in vivo. *Acta Physiol., 217*(2), 152-163.

Hamner, S. R., Seth, A., & Delp, S. L. (2010). Muscle contributions to propulsion and support during running. *J. Biomech., 43*(14), 2709-2716.

Hawkins, D., & Hull, M. L. (1990). A method for determining lower extremity muscle-tendon lengths during flexion/extension movements. *J. Biomech., 23*(5), 487-494.

Hermens, H. J., Freriks, B., Disselhorst-Klug, C., & Rau, G. (2000). Development of recommendations for SEMG sensors and sensor placement procedures. *J. Electromyogr. Kinesiol., 10*(5), 361-374.

Herzog, W. (1998). History dependence of force production in skeletal muscle: a proposal for mechanisms. *J. Electromyogr. Kinesiol., 8*(2), 111-117.

Herzog, W. (2018). The multiple roles of titin in muscle contraction and force production. *Biophys. Rev.*, 1-13.

Herzog, W., Duvall, M., & Leonard, T. R. (2012). Molecular mechanisms of muscle force regulation: a role for titin? *Exerc. Sport Sci. Rev., 40*(1), 50-57.

Herzog, W., Read, L. J., & ter Keurs, H. E. D. J. (1991). Experimental determination of force—length relations of intact human gastrocnemius muscles. *Clinical Biomechanics, 6*(4), 230-238.

Herzog, W., Schappacher, G., DuVall, M., Leonard, T. R., & Herzog, J. A. (2016). Residual Force Enhancement Following Eccentric Contractions: A New Mechanism Involving Titin. *Physiology, 31*(4), 300.

Hess, G. P., Cappiello, W. L., Poole, R. M., & Hunter, S. C. (1989). Prevention and Treatment of Overuse Tendon Injuries. *Sports Med., 8*(6), 371-384.

Hill, A. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. Lond. B. Biol. Sci., 126*(843), 136-195.

Hill, A. V. (1964). The Efficiency of Mechanical Power Development during Muscular Shortening and Its Relation to Load. *Proc. R. Soc. Lond., B, Biol. Sci., 159*(975), 319-324.

Hirayama, K., Iwanuma, S., Ikeda, N., Yoshikawa, A., Ema, R., & Kawakami, Y. (2017). Plyometric training favors optimizing muscle–tendon behavior during depth jumping. *Front. Physiol., 8*, 16.

Hof, A. L., Geelen, B. A., & Van den Berg, J. (1983). Calf muscle moment, work and efficiency in level walking; Role of series elasticity. *J. Biomech., 16*(7), 523-537.

Hollville, E., Nordez, A., Guilhem, G., Lecompte, J., & Rabita, G. (2018). Interactions between fascicles and tendinous tissues in gastrocnemius medialis and vastus lateralis during drop landing. *Scand. J. Med. Sci. Sports, 0*(ja).

Holt, N. C., Danos, N., Roberts, T. J., & Azizi, E. (2016). Stuck in gear: age-related loss of variable gearing in skeletal muscle. *J. Exp. Biol., 219*(7), 998.

Houghton, L. A., Dawson, B. T., & Rubenson, J. (2013). Effects of Plyometric Training on Achilles Tendon Properties and Shuttle Running During a Simulated Cricket Batting Innings. *J. Strength Cond. Res., 27*(4).

Huxley, A. F., & Niedergerke, R. (1954). Structural changes in muscle during contraction: interference microscopy of living muscle fibres. *Nature, 173*(4412), 971.

Huxley, H. E. (1953). X-ray analysis and the problem of muscle. *Proc. R. Soc. Lond. B. Biol. Sci., 141*(902), 59.

Huxley, H. E. (1957). The double array of filaments in cross-striated muscle. *J. Biophys. Biochem. Cytol., 3*(5), 631.

Huxley, H. E. (1969). The mechanism of muscular contraction. *Science, 164*(3886), 1356-1365.

Ikai, M., & Fukunaga, T. (1968). Calculation of muscle strength per unit cross-sectional area of human muscle by means of ultrasonic measurement. *Int. Z. Angew. Physiol., 26*(1), 26-32.

Ishikawa, M., & Komi, P. V. (2007). The role of the stretch reflex in the gastrocnemius muscle during human locomotion at various speeds. *J. Appl. Physiol., 103*(3), 1030-1036.

Ishikawa, M., Niemelä, E., & Komi, P. V. (2005). Interaction between fascicle and tendinous tissues in short-contact stretch-shortening cycle exercise with varying eccentric intensities. *J. Appl. Physiol., 99*(1), 217-223.

Kawakami, Y., Abe, T., Kuno, S.-Y., & Fukunaga, T. (1995). Training-induced changes in muscle architecture and specific tension. *Eur. J. Appl. Physiol. Occup. Physiol., 72*(1), 37-43.

Kaya, M., Jinha, A., Leonard, T. R., & Herzog, W. (2005). Multi-functionality of the cat medical gastrocnemius during locomotion. *J. Biomech., 38*(6), 1291-1301.

Ker, R. F. (1981). Dynamic tensile properties of the plantaris tendon of sheep (Ovis aries). *J. Exp. Biol., 93*(1), 283.

Kim, S. Y., Ko, J. B., Farthing, J. P., & Butcher, S. J. (2015). Investigation of supraspinatus muscle architecture following concentric and eccentric training. *J. Sci. Med. Sport, 18*(4), 378-382.

Kongsgaard, M., Reitelseder, S., Pedersen, T. G., Holm, L., Aagaard, P., Kjær, M., & Magnusson, S. P. (2007). Region specific patellar tendon hypertrophy in humans following resistance training. *Acta Physiol., 191*(2), 111-121.

Konow, N., Azizi, E., & Roberts, T. J. (2012). Muscle power attenuation by tendon during energy dissipation. *Proc. R. Soc. Lond. B. Biol. Sci., 279*(1731), 1108.

Konow, N., & Roberts, T. J. (2015). The series elastic shock absorber: tendon elasticity modulates energy dissipation by muscle during burst deceleration. *Proc. R. Soc. Lond. B. Biol. Sci., 282*(1804).

Kubo, K., Ikebukuro, T., Maki, A., Yata, H., & Tsunoda, N. (2012). Time course of changes in the human Achilles tendon properties and metabolism during training and detraining in vivo. *Eur. J. Appl. Physiol., 112*(7), 2679-2691.

Kubo, K., Ikebukuro, T., Yata, H., Tsunoda, N., & Kanehisa, H. (2010a). Effects of Training on Muscle and Tendon in Knee Extensors and Plantar Flexors in Vivo. *J. Appl. Biomech., 26*(3), 316-323.

Kubo, K., Ishigaki, T., & Ikebukuro, T. (2017). Effects of plyometric and isometric training on muscle and tendon stiffness in vivo. *Physiol. Rep., 5*(15).

Kubo, K., Kanehisa, H., & Fukunaga, T. (2002). Effects of resistance and stretching training programmes on the viscoelastic properties of human tendon structures in vivo. *J. Physiol., 538*(Pt 1), 219-226.

Kubo, K., Miyazaki, D., Shimoju, S., & Tsunoda, N. (2015). Relationship between elastic properties of tendon structures and performance in long distance runners. *Eur. J. Appl. Physiol., 115*(8), 1725-1733.

Kubo, K., Tabata, T., Ikebukuro, T., Igarashi, K., Yata, H., & Tsunoda, N. (2010b). Effects of mechanical properties of muscle and tendon on performance in long distance runners. *Eur. J. Appl. Physiol., 110*(3), 507-514.

Kubo, K., Teshima, T., Hirose, N., & Tsunoda, N. (2014). A Cross-Sectional Study of the Plantar Flexor Muscle and Tendon during Growth. *Int. J. Sports Med., 35*(10), 828-834.

Kurokawa, S., Fukunaga, T., & Fukashiro, S. (2001). Behavior of fascicles and tendinous structures of human gastrocnemius during vertical jumping. *J. Appl. Physiol., 90*(4), 1349-1358.

Kwah, L. K., Pinto, R. Z., Diong, J., & Herbert, R. D. (2013). Reliability and validity of ultrasound measurements of muscle fascicle length and pennation in humans: a systematic review. *J. Appl. Physiol., 114*(6), 761-769.

Kyröläinen, H., Avela, J., & Komi, P. V. (2005). Changes in muscle activity with increasing running speed. *J. Sports Sci., 23*(10), 1101-1109.

Lai, A., Lichtwark, G. A., Schache, A. G., Lin, Y.-C., Brown, N. A. T., & Pandy, M. G. (2015). In vivo behavior of the human soleus muscle with increasing walking and running speeds. *J. Appl. Physiol., 118*(10), 1266-1275.

Lai, A., Schache, A. G., Lin, Y.-C., & Pandy, M. G. (2014). Tendon elastic strain energy in the human ankle plantar-flexors and its role with increased running speed. *J. Exp. Biol., 217*(17), 3159-3168.

Lai, A. K. M., Lichtwark, G. A., Schache, A. G., & Pandy, M. G. (2018). Differences in in vivo muscle fascicle and tendinous tissue behavior between the ankle plantarflexors during running. *Scand. J. Med. Sci. Sports, 28*(7), 1828-1836.

Lichtwark, G. A., Bougoulias, K., & Wilson, A. M. (2007). Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. *J. Biomech., 40*(1), 157-164.

Lichtwark, G. A., Farris, D. J., Chen, X., Hodges, P. W., & Delp, S. L. (2018). Microendoscopy reveals positive correlation in multiscale length changes and variable sarcomere lengths across different regions of human muscle. *J. Appl. Physiol.*

Lichtwark, G. A., & Wilson, A. M. (2005a). Effects of series elasticity and activation conditions on muscle power output and efficiency. *J. Exp. Biol., 208*(15), 2845.

Lichtwark, G. A., & Wilson, A. M. (2005b). In vivo mechanical properties of the human Achilles tendon during one-legged hopping. *J. Exp. Biol., 208*(24), 4715-4725.

Lichtwark, G. A., & Wilson, A. M. (2006). Interactions between the human gastrocnemius muscle and the Achilles tendon during incline, level and decline locomotion. *J. Exp. Biol., 209*(21), 4379-4388.

Lichtwark, G. A., & Wilson, A. M. (2007). Is Achilles tendon compliance optimised for maximum muscle efficiency during locomotion? *J. Biomech., 40*(8), 1768-1775.

Lichtwark, G. A., & Wilson, A. M. (2008). Optimal muscle fascicle length and tendon stiffness for maximising gastrocnemius efficiency during human walking and running. *J. Theor. Biol., 252*(4), 662-673.

Lieber, R. L., & Brown, C. G. (1992). Quantitative method for comparison of skeletal muscle architectural properties. *J. Biomech., 25*(5), 557-560.

Lieber, R. L., & Fridén, J. (2000). Functional and clinical significance of skeletal muscle architecture. *Muscle Nerve, 23*(11), 1647-1666.

Maganaris, C. N. (2003a). Force-length characteristics of the in vivo human gastrocnemius muscle. *Clin. Anat., 16*(3), 215-223.

Maganaris, C. N. (2003b). Tendon conditioning: artefact or property? *Proc. R. Soc. Lond. B. Biol. Sci., 270*(Suppl 1), S39.

Maganaris, C. N., Kawakami, Y., & Fukunaga, T. (2001). Changes in aponeurotic dimensions upon muscle shortening: in vivo observations in man. *J. Anat., 199*(4), 449-456.

Maganaris, C. N., & Paul, J. P. (1999). In vivo human tendon mechanical properties. *J. Physiol., 521*(1), 307-313.

Maganaris, C. N., & Paul, J. P. (2000). Hysteresis measurements in intact human tendon. *J. Biomech., 33*(12), 1723-1727.

Magnusson, S. P., Hansen, P., Aagaard, P., Brønd, J., Dyhre-Poulsen, P., Bojsen-Moller, J., & Kjaer, M. (2003). Differential strain patterns of the human gastrocnemius aponeurosis and free tendon, in vivo. *Acta Physiol. Scand., 177*(2), 185-195.

Mahieu, N. N., McNair, P., Cools, A., D'Haen, C., Vandermeulen, K., & Witvrouw, E. (2008). Effect of eccentric training on the plantar flexor muscle-tendon tissue properties. *Med. Sci. Sports Exerc., 40*(1), 117-123.

Massey, G. J., Balshaw, T. G., Maden-Wilkinson, T. M., Tillin, N. A., & Folland, J. P. (2018). Tendinous Tissue Adaptation to Explosive- vs. Sustained-Contraction Strength Training. *Front. Physiol., 9*, 1170.

Matsubara, I., & Elliott, G. F. (1972). X-ray diffraction studies on skinned single fibres of frog skeletal muscle. *J. Mol. Biol., 72*(3), 657-669.

McGowan, C. P., Duarte, H. A., Main, J. B., & Biewener, A. A. (2006). Effects of load carrying on metabolic cost and hindlimb muscle dynamics in guinea fowl (Numida meleagris). *J. Appl. Physiol., 101*(4), 1060-1069.

Narici, M. V., Binzoni, T., Hiltbrand, E., Fasel, J., Terrier, F., & Cerretelli, P. (1996). In vivo human gastrocnemius architecture with changing joint angle at rest and during graded isometric contraction. *J. Physiol., 496*(1), 287-297.

Narici, M. V., Roi, G. S., Landoni, L., Minetti, A. E., & Cerretelli, P. (1989). Changes in force, cross-sectional area and neural activation during strength training and detraining of the human quadriceps. *Eur. J. Appl. Physiol. Occup. Physiol., 59*(4), 310-319.

Obst, S. J., Barber, L., Miller, A., & Barrett, R. S. (2017). Reliability of Achilles Tendon Moment Arm Measured In Vivo Using Freehand Three-Dimensional Ultrasound. *J. Appl. Biomech., 33*(4), 300-304.

Pollock, C. M., & Shadwick, R. E. (1994). Relationship between body mass and biomechanical properties of limb tendons in adult mammals. *Am. J. Physiol. Regul. Integr. Comp. Physiol., 266*(3), R1016-R1021.

Potier, T. G., Alexander, C. M., & Seynnes, O. R. (2009). Effects of eccentric strength training on biceps femoris muscle architecture and knee joint range of movement. *Eur. J. Appl. Physiol., 105*(6), 939-944.

Proske, U., & Morgan, D. L. (2001). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J. Physiol., 537*(2), 333-345.

Raiteri, B. J. (2018). Aponeurosis behaviour during muscular contraction: A narrative review. *Eur. J. Sport Sci.*, 1-11.

Raiteri, B. J., Cresswell, A. G., & Lichtwark, G. A. (2016). Three-dimensional geometrical changes of the human tibialis anterior muscle and its central aponeurosis measured with three-dimensional ultrasound during isometric contractions. *PeerJ, 4,* e2260.

Raiteri, B. J., Cresswell, A. G., & Lichtwark, G. A. (2018). Muscle-tendon length and force affect human tibialis anterior central aponeurosis stiffness in vivo. *PNAS*.

Rana, M., Hamarneh, G., & Wakeling, J. M. (2014). 3D curvature of muscle fascicles in triceps surae. *J. Appl. Physiol., 117*(11), 1388-1397.

Randhawa, A., Jackman, M. E., & Wakeling, J. M. (2013). Muscle gearing during isotonic and isokinetic movements in the ankle plantarflexors. *Eur. J. Appl. Physiol., 113*(2), 437-447.

Reeves, N. D., Maganaris, C. N., Longo, S., & Narici, M. V. (2009). Differential adaptations to eccentric versus conventional resistance training in older humans. *Exp. Physiol., 94*(7), 825-833.

Roberts, T. J., & Azizi, E. (2010). The series-elastic shock absorber: tendons attenuate muscle power during eccentric actions. *J. Appl. Physiol., 109*(2), 396-404.

Roberts, T. J., & Azizi, E. (2011). Flexible mechanisms: the diverse roles of biological springs in vertebrate movement. *J. Exp. Biol., 214*(3), 353-361.

Roberts, T. J., & Konow, N. (2013). How tendons buffer energy dissipation by muscle. *Exerc. Sport Sci. Rev., 41*(4).

Roberts, T. J., & Marsh, R. L. (2003). Probing the limits to muscle-powered accelerations: lessons from jumping bullfrogs. *J. Exp. Biol., 206*(15), 2567.

Roberts, T. J., Marsh, R. L., Weyand, P. G., & Taylor, C. R. (1997). Muscular Force in Running Turkeys: The Economy of Minimizing Work. *Science, 275*(5303), 1113-1115.

Rubenson, J., Pires, N. J., Loi, H. O., Pinniger, G. J., & Shannon, D. G. (2012). On the ascent: the soleus operating length is conserved to the ascending limb of the force–length curve across gait mechanics in humans. *J. Exp. Biol., 215*(20), 3539-3551.

Rutherford, O. M., & Jones, D. A. (1992). Measurement of fibre pennation using ultrasound in the human quadriceps in vivo. *Eur. J. Appl. Physiol. Occup. Physiol., 65*(5), 433-437.

Seynnes, O. R., Bojsen-Møller, J., Albracht, K., Arndt, A., Cronin, N. J., Finni, T., & Magnusson, S. P. (2015). Ultrasound-Based Testing Of Tendon Mechanical Properties: A Critical Evaluation. *J. Appl. Physiol., 18*(2), 133-141.

Seynnes, O. R., de Boer, M., & Narici, M. V. (2007). Early skeletal muscle hypertrophy and architectural changes in response to high-intensity resistance training. *J. Appl. Physiol., 102*(1), 368.

Seynnes, O. R., Erskine, R. M., Maganaris, C. N., Longo, S., Simoneau, E. M., Grosset, J. F., & Narici, M. V. (2009). Training-induced changes in structural and mechanical properties of the patellar tendon are related to muscle hypertrophy but not to strength gains. *J. Appl. Physiol., 107*(2), 523-530.

Sharifnezhad, A., Marzilger, R., & Arampatzis, A. (2014). Effects of load magnitude, muscle length and velocity during eccentric chronic loading on the longitudinal growth of the vastus lateralis muscle. *J. Exp. Biol., 217*(15), 2726.

Spanjaard, M., Reeves, N. D., van Dieën, J. H., Baltzopoulos, V., & Maganaris, C. N. (2007). Gastrocnemius muscle fascicle behavior during stair negotiation in humans. *J. Appl. Physiol., 102*(4), 1618-1623.

Spanjaard, M., Reeves, N. D., van Dieën, J. H., Baltzopoulos, V., & Maganaris, C. N. (2008). Lower-limb biomechanics during stair descent: influence of step-height and body mass. *J. Exp. Biol., 211*(9), 1368-1375.

Stafilidis, S., & Arampatzis, A. (2007). Muscle – tendon unit mechanical and morphological properties and sprint performance. *J. Sports Sci., 25*(9), 1035-1046.

Stosic, J., & Finni, T. (2011). Gastrocnemius tendon length and strain are different when assessed using straight or curved tendon model. *Eur. J. Appl. Physiol., 111*(12), 3151-3154.

Tilp, M., Steib, S., & Herzog, W. (2012). Length changes of human tibialis anterior central aponeurosis during passive movements and isometric, concentric, and eccentric contractions. *Eur. J. Appl. Physiol., 112*(4), 1485-1494.

Timmins, R. G., Shield, A. J., Williams, M. D., Lorenzen, C., & Opar, D. A. (2016). Architectural adaptations of muscle to training and injury: a narrative review outlining the contributions by fascicle length, pennation angle and muscle thickness. *Br. J. Sports Med., 50*(23), 1467.

Tipton, C. M., Matthes, R. D., Maynard, J. A., & Carey, R. A. (1975). The influence of physical activity on ligaments and tendons. *Med. Sci. Sports, 7*(3), 165-175.

Valamatos, M. J., Tavares, F., Santos, R. M., Veloso, A. P., & Mil-Homens, P. (2018). Influence of full range of motion vs. equalized partial range of motion training on muscle architecture and mechanical properties. *Eur. J. Appl. Physiol.*

Waugh, C. M., Alktebi, T., Sa, A., & Scott, A. (2017). Impact of rest duration on Achilles tendon structure and function following isometric training. *Scand. J. Med. Sci. Sports, 28*(2), 436-445.

Waugh, C. M., Korff, T., Fath, F., & Blazevich, A. J. (2013). Rapid force production in children and adults: mechanical and neural contributions. *Med. Sci. Sports Exerc., 45*(4), 762-771.

Waugh, C. M., Korff, T., Fath, F., & Blazevich, A. J. (2014). Effects of resistance training on tendon mechanical properties and rapid force production in prepubertal children. *J. Appl. Physiol., 117*(3), 257.

Wernbom, M., Augustsson, J., & Thomeé, R. (2007). The Influence of Frequency, Intensity, Volume and Mode of Strength Training on Whole Muscle Cross-Sectional Area in Humans. *Sports Med., 37*(3), 225-264.

Wickiewicz, T. L., Roy, R. R., Powell, P. L., & Edgerton, V. R. (1983). Muscle Architecture of the Human Lower Limb. *Clin. Orthop. Relat. Res., 179.*

Wiesinger, H.-P., Kösters, A., Müller, E., & Seynnes, O. R. (2015). Effects of Increased Loading on In Vivo Tendon Properties: A Systematic Review. *Med. Sci. Sports Exerc., 47*(9), 1885-1895.

Wiesinger, H.-P., Rieder, F., Kösters, A., Müller, E., & Seynnes, O. R. (2016). Are Sport-Specific Profiles of Tendon Stiffness and Cross-Sectional Area Determined by Structural or Functional Integrity? *PLoS One, 11*(6), e0158441.

Wiesinger, H.-P., Rieder, F., Kösters, A., Müller, E., & Seynnes, O. R. (2017). Sport-Specific Capacity to Use Elastic Energy in the Patellar and Achilles Tendons of Elite Athletes. *Front. Physiol., 8*, 132.

Wilson, A. M., Watson, J. C., & Lichtwark, G. A. (2003). A catapult action for rapid limb protraction. *Nature, 421*, 35.

Wu, Y. K., Lien, Y. H., Lin, K. H., Shih, T. T. F., Wang, T. G., & Wang, H. K. (2010). Relationships between three potentiation effects of plyometric training and performance. *Scand. J. Med. Sci. Sports, 20*(1), e80-e86.

Zajac, F. E. (1989). Muscle and tendon: properties, models, scaling, and application to biomechanics and motor control. *Crit. Rev. Biomed. Eng., 17*(4), 359-411.

Zelik, K. E., & Franz, J. R. (2017). It's positive to be negative: Achilles tendon work loops during human locomotion. *PLoS One, 12*(7), e0179976.

Zelik, K. E., & Honert, E. C. (2018). Ankle and foot power in gait analysis: Implications for science, technology and clinical assessment. *J. Biomech.*

Article 1

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RESEARCH ARTICLE

Modulation of muscle–tendon interaction in the human triceps surae during an energy dissipation task

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ABSTRACT

The compliance of elastic elements allows muscles to dissipate energy safely during eccentric contractions. This buffering function is well documented in animal models but our understanding of its mechanism in humans is confined to non-specific tasks, requiring a subsequent acceleration of the body. The present study aimed to examine the behaviour of the human triceps surae muscle–tendon unit (MTU) during a pure energy dissipation task, under two loading conditions. Thirty-nine subjects performed a single-leg landing task, with and without added mass. Ultrasound measurements were combined with three-dimensional kinematics and kinetics to determine instantaneous length changes of MTUs, muscle fascicles, Achilles tendon and combined elastic elements. Gastrocnemius and soleus MTUs lengthened during landing. After a small concentric action, fascicles contracted eccentrically during most of the task, whereas plantar flexor muscles were activated. Combined elastic elements lengthened until peak ankle moment and recoiled thereafter, whereas no recoil was observed for the Achilles tendon. Adding mass resulted in greater negative work and MTU lengthening, which were accompanied by a greater stretch of tendon and elastic elements and a greater recruitment of the soleus muscle, without any further fascicle strain. Hence, the buffering action of elastic elements delimits the maximal strain and lengthening velocity of active muscle fascicles and is commensurate with loading constraints. In the present task, energy dissipation was modulated via greater MTU excursion and more forceful eccentric contractions. The distinct strain pattern of the Achilles tendon supports the notion that different elastic elements may not systematically fulfil the same function.

KEY WORDS: Achilles tendon, Energy absorption, Mechanical buffer

INTRODUCTION

The spring-like properties of elastic elements, i.e. tendinous or connective tissue, enhance the function of muscle–tendon units (MTU) of the lower limb in a variety of movements (Roberts and Azizi, 2011). During running, the body's potential energy is converted to elastic energy, which is stored in elastic elements and released to enhance movement efficiency (Fukunaga et al., 2001; Lichtwark and Wilson, 2006). During jumping, power output is increased by a rapid release of energy stored in elastic structures during muscle contraction (Farris et al., 2016; Kurokawa et al., 2001). Hence, the temporary storage of energy in elastic structures enables the uncoupling of muscle work from joint movement.

Evidence from animal studies indicates that tasks where energy dissipation is required may also benefit from such a mechanism. Using isolated preparations of plantar flexor muscles from cats and turkeys, respectively, Griffiths (1991) and Roberts and Azizi (2010) have shown that rapid stretches of the MTU were taken up by elongation of the tendon. These *in situ* observations and subsequent *in vivo* studies have convincingly demonstrated that elastic structures provide a buffering mechanism that attenuates negative power input to the muscle. As shown during drop landings of turkeys, most of the lengthening of the MTU is taken up by tendon elongation, effectively delaying and slowing down fascicle lengthening to actively dissipate energy (Konow et al., 2012). Konow and Roberts (2015) attributed this mechanism to the double advantage of constraining the muscle to a favourable operating length and to a safer lengthening velocity. Despite the importance of such a mechanical buffer in daily activities where deceleration is required (e.g. walking down stairs or downhill, dropping from a ledge), energy dissipation does not seem to have been investigated systematically in humans.

A few studies have provided insight into the role of muscle and elastic elements when energy is dissipated in the human lower leg during locomotor tasks. These movements typically involve an initial deceleration of the body, followed by an acceleration in a different direction (e.g. countermovement jumps and stair descent). Kawakami et al. (2002) found that, during the ankle dorsiflexion phase of a countermovement jump, gastrocnemius fascicles were passively lengthened for a short time before contracting isometrically, whereas the MTU lengthened. During stair descent, Spanjaard et al. (2007) reported that the fascicles of the gastrocnemius muscle shortened at first when ground reaction force (GRF) increased and lengthened thereafter throughout the single-support phase, whereas the MTU acted relatively isometrically. Thus, the deceleration required in these examples results in an uncoupling of muscle fascicle behaviour from that of the MTU, consistent with the buffering mechanism shown in animal studies. However, the subsequent acceleration inherent in such movements implies complex muscle–tendon behaviour, whereby energy is partly dissipated and partly recycled to limit muscle work or to enhance mechanical power during the push-off phase. For instance, the relatively low muscle activity recorded during fascicle lengthening may reflect the necessity to limit energy dissipation when descending stairs. Consequently, the task of stair descent may

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not be optimal to investigate the role of muscle and elastic elements during energy dissipation. Additionally, the role of different elastic elements is unclear in the mentioned studies. Lichtwark and Wilson (2006) showed that, during walking and running, the strain of so-called 'series elastic elements' can considerably differ from that of the Achilles tendon *per se*.

Hence, the objective of this study was to investigate the behaviour of muscle, tendon and elastic elements of the lower leg during a pure energy dissipation task. During a one-legged step landing, we predicted that elastic elements would buffer the rapid stretch of the triceps surae MTU, enabling muscle fascicles to operate at a limited range and velocity, but at a higher level of muscle activity than during locomotion. The stability of this mechanism under higher requirements for energy dissipation was tested in an additional experimental condition with added mass. Assuming an increase in energy absorption requirements, we expected an increase in the strain of the fascicles and/or an increase in force, which would be reflected by increased electromyographic (EMG) activity. Based on differences in fascicle behaviour observed between the biarticular gastrocnemius and the monoarticular soleus during locomotion (Cronin et al., 2013), both muscles were included in the analysis. Being monoarticular, the soleus muscle was expected to be more affected than the gastrocnemius by increased ankle flexion under the added-mass condition and to display more pronounced changes (e.g. increases in fascicle strain and/or EMG activity). A secondary objective was to elucidate more specifically the role of the Achilles tendon during energy dissipation. As previously shown, the strain estimated during walking and running for so-called 'series elastic elements' (essentially Achilles tendon, aponeurosis and proximal tendon) can considerably differ from that of the Achilles tendon *per se* (Lichtwark and Wilson, 2006). We therefore measured length changes of the Achilles tendon and compared it to the calculated strain of combined elastic elements. Owing to their different mechanical properties, we expected distinctive strain patterns in the tendon and in combined elastic elements during energy dissipation, despite these structures often being referred to interchangeably.

MATERIALS AND METHODS

Subjects

Thirty-nine male athletes habitually engaged in ski jumping (n=21, age 23±3 years, height 179±6 cm and mass 64±4 kg) and distance running (n=18, age 27±5 years, height 180±5 cm and mass 68±6 kg) took part in this study. The two types of athletes were initially recruited for a larger project. They were merged for this study after verifying the homogeneity of their anthropometric characteristics (age 25±4 years, height 179±6 cm and mass 66±5 kg) and the similarities of their triceps surae MTU properties. All subjects gave their written informed consent and the ethical committee of the Norwegian School of Sport Sciences approved the study.

Protocol

Resting anatomical measurements were performed prior to testing. Height, body mass and right lower leg length (between the lateral malleolus and the lateral femur epicondyle) were measured for each subject. While the subjects were lying prone with the ankle joint at 90 deg (anatomical position), ultrasound images were recorded (LS128, Telemed, Vilnius, Lithuania) to visualise the muscle architecture of the gastrocnemius medialis and soleus.

After a general warm up consisting of 5 min running on a treadmill at a self-selected speed, subjects were then asked to perform a unilateral step landing task. The task was performed from a step height adjusted to individual body mass to standardise potential energy (240 J). Subjects were instructed to step down with their right leg whilst the left foot remained on the step to maintain balance. Five trials were performed to record ultrasound scans of the gastrocnemius medialis fascicles, and five additional trials were used to scan the gastrocnemius myotendinous junction. The size and strapping of the ultrasound probe did not allow simultaneous positioning of EMG sensors over the target muscles. For this reason, gastrocnemius, soleus and tibialis anterior EMGs were recorded from the left leg in five separate trials. The 15 landing trials were conducted twice, once with body mass only and once with a vest loaded with 20% individual body mass.

Kinematics and kinetics

During the step landing task, motion capture (12 cameras, Qualisys, Gothenburg, Sweden) was used to record the positions of 18 reflective markers placed on the right leg and the hips. A modified Cleveland marker set was used for the right lower extremities. Markers were placed on the left and right anterior and posterior iliac spines to define the pelvis segment and hip joint centres (Bell et al., 1989). The right knee joint centre was defined as the mid-distance between the markers on the medial and lateral condyles. Likewise, the ankle joint centre was determined as the mid-point between the medial and lateral malleoli. To track the foot segment, markers were placed on the calcaneus and the metatarsals (first, second and fifth head). The thigh and shank segments were tracked with marker clusters consisting of four markers, placed mid-way on their respective lateral sides. Force data were recorded on a force plate (M-Gait, Motekforce Link, Amsterdam, The Netherlands) at a frequency of 1500 Hz.

Kinematic and kinetic data were analysed offline using a standard Newton–Euler inverse dynamics procedure (Visual 3D, C-Motion Inc., Germantown, MD, USA). Joint angles, moments and powers were expressed in the coordinate system of the respective proximal segment. Reference frames for all segments were defined during a standing reference measurement. Joint power was calculated as the product of the joint moment and the joint angular velocity. Data were filtered with a bidirectional first-order low-pass Butterworth filter with a cut-off frequency of 15 Hz (Kristianslund et al., 2012). Shank length was used in combination with ankle and knee joint angles to estimate MTU lengths of the gastrocnemius and soleus based on a frequently used regression equation (Hawkins and Hull, 1990). Strain velocities of MTUs were determined by differentiating their respective lengths.

Muscle–tendon behaviour

Muscle fascicles were imaged using ultrasound at a frame frequency of 80 Hz. The transducer was positioned over the gastrocnemius and soleus muscle bellies to visualize fascicles and aponeuroses. The transducer was securely fastened to the skin with adhesive tape at the interface of a custom-made holder to avoid probe movement. Fascicle length and pennation angle were analysed using a semi-automated tracking algorithm (Cronin et al., 2011; Farris and Lichtwark, 2016). Fascicle length was defined as the length between the insertions to the superficial and deep aponeuroses. Pennation angle was defined as the angle between fascicles and the deep aponeurosis for gastrocnemius, and as the angle between fascicles and the superficial aponeurosis for the soleus. The compound length of series elastic elements was calculated as follows (Fukunaga et al., 2001):

$$L_{\mathrm{EE}} = L_{\mathrm{MTU}} - L_{\mathrm{f}} \cdot \cos\alpha, \qquad (1)$$

where L_{EE} is the length of the series elastic elements, L_{MTU} is the length of the MTU, L_{f} is the length of the fascicle and α is the fascicle pennation angle. Velocities were calculated as the first derivatives of MTU and fascicle lengthening.

Ultrasound scans of the gastrocnemius medialis myotendinous junction in the line of action of the muscle were used to estimate tendon length. Three reflective markers forming a triangle were rigidly attached to the ultrasound transducer, which was secured to the skin. Marker positions were synchronously recorded with the Qualisys motion-capture system. The position calibration of the transducer markers in relation to the ultrasound image plane allowed the calculation of position and orientation of the ultrasound image in the three-dimensional laboratory coordinate system (Lichtwark and Wilson, 2005). The displacement of the myotendinous junction was analysed in the two-dimensional (2D) ultrasound image using Tracker software (Tracker 4.95; www.physlets.org/tracker/). Achilles tendon length was estimated as the distance between the myotendinous junction and the calcaneus marker placed over the osteotendinous junction, as determined with ultrasound scanning. All ultrasound data were filtered similarly to kinematic and kinetic data.

Electromyography

Gastrocnemius, soleus and tibialis anterior EMGs were recorded from the left leg (TeleMyo DTS, Noraxon U.S.A. Inc., Scottsdale, AZ, USA) at an acquisition frequency of 1500 Hz. Electrode positioning and skin preparation were performed according to the SENIAM guidelines (Hermens et al., 2000). Data were treated offline with a bidirectional high-pass Butterworth filter with a cut-off frequency of 20 Hz. After rectifying, the signal was filtered with a 6 Hz bidirectional low-pass Butterworth filter (Maharaj et al., 2016). Within each subject, EMG data were normalised to the peak value reached during the step landing task without additional mass. Mean EMG values were calculated over the five trials of each subject. In some instances, one of the trials contained data points deviating from the mean by more than three times the s.d. These trials were excluded and the mean was re-calculated with the remaining four trials.

Data processing and statistical analysis

Data were reduced to the landing phase corresponding to the generation of negative ankle power and resampled to 101 data points. In the case of EMG data recorded from the left leg, kinematic data were not available to identify the phase of negative power. Therefore, the landing phase was defined for this variable by matching GRF events recorded in both legs. For all variables, the analysed phase was divided into three sub-phases based on GRF (Fig. 1): near constant force (phase 1), force rise (phase 2) and force decay (phase 3). Phase 1 started at the onset of negative ankle power and ended at the point at which the fastest change in rate of GRF development occurred (as defined by the peak of the GRF second derivative). Although force arguably increases throughout phases 1 and 2, the event closing the first phase was defined to offer an additional level of analysis because it also coincides with a change in the lengthening patterns of the MTU and its components. The subsequent phase 2 ended at the time point of peak GRF. The third phase was defined as the remaining time period, where GRF decreased.

For each subject, a multiple-correlation analysis was performed between the time–ankle-angle curves to identify atypical trials. A correlation coefficient of ≥0.95 was required between at least four of the trials to ensure that retained data would reflect a consistent execution of the task for each individual. Additionally, to ensure tracking quality, a coefficient of correlation above 0.90 for a given trial was required for inclusion. All data were analysed as the mean of at least four trials for each subject.

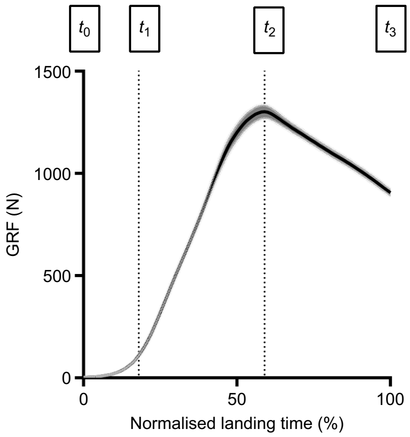

Fig. 1. Ground reaction force (GRF) during the energy dissipation task. All data were obtained between t_0 (0% normalised landing time) and t_3 (100% normalised landing time), corresponding to the onset and the end of negative ankle power, respectively. Three sub-phases [near constant force (phase 1), force rise (phase 2) and force decay (phase 3)] were determined by calculating t_1 (maximum change in rate of force development) and t_2 (peak force).

For statistical analysis, repeated measures one-way ANOVAs and Tukey's multiple comparisons were used to compare changes in length of the different MTU constituents during phases 1, 2 and 3 (t_0 to t_1, t_1 to t_2 and t_2 to t_3, respectively) (Prism, GraphPad Software Inc., La Jolla, CA, USA). Student's *t*-tests were performed to compare the changes in MTU and fascicle length between gastrocnemius and soleus muscles, and between ankle and knee moment and power. To analyse the influence of mass conditions (body mass versus added mass), relevant peak values and changes occurring in the three phases were compared using *t*-tests. The alpha level was set to 0.05. The data are presented as means±s.d. in the text, tables and boxplots, and as means±s.e.m. in the figures with line plots for clarity.

RESULTS

The data sets recorded during the condition with added mass had to be discarded for two subjects whose ankle kinematics data did not meet the inclusion criteria (i.e. changes in ankle joint angle differed too much between trials). In addition, the soleus ultrasound data of four subjects were removed due to insufficient quality of the images of the fascicles. Hence, the data of 39 subjects were included in the final analysis of the task with body mass, 37 subjects for the task with added mass. For the variables obtained from soleus scans (length and velocity of soleus fascicle and MTU), the number of subjects in both conditions was 35 and 33, respectively.

Kinematics and kinetics

Ankle and knee joint angle, moment and power measured during the landing phase are presented in Fig. 2. Peak moment and power normalised to body mass were higher in the ankle joint (-1.79 ± 0.29 N m kg^{-1}; -10.89 ± 2.36 W kg^{-1}) than in the knee joint (-1.33 ± 0.50 N m kg^{-1}, $P\leq0.01$; -4.92 ± 3.09 W kg^{-1}, $P\leq0.01$) and occurred earlier in the ankle than in the knee joint.

In the condition with added mass, ankle dorsiflexion and knee flexion were greater than in the unloaded condition. For both joints,

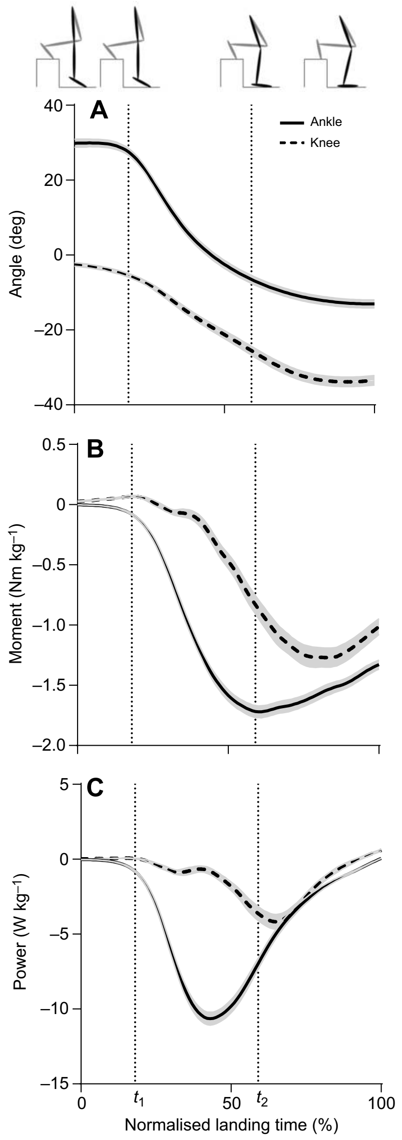

Fig. 2. Joint angle, normalised moment and normalised power at the ankle and knee joints during energy absorption in the body-mass condition. Data are means±s.e.m. Time series are normalised to 101 points. Negative changes in angle correspond to ankle dorsiflexion and knee flexion (0 deg corresponds to anatomical position). Moment and power are normalised to body mass. Vertical dotted lines represent the time points of the highest rate of GRF development (t_1) and peak GRF (t_2).

significantly higher peak moments and powers were produced with added mass (Fig. 3A–E). Adding mass resulted in an increase in negative work at the ankle joint from −58.06±13.03 to −67.06±15.08 J and the duration of the landing phase was prolonged by 10% (0.21±0.02 s without, versus 0.24±0.02 s with added mass).

Muscle activity

The EMG signals obtained from gastrocnemius, soleus and tibialis anterior muscles were normalised to peak values obtained during the phase of negative power, without added mass (Fig. 4). All three muscles were activated during the negative power production period. The greatest activity of gastrocnemius was seen during phase 1, whereas soleus activity increased throughout phases 1 and 2. Additional mass increased the activity of soleus and tibialis anterior, but not that of gastrocnemius (Fig. 5).

MTU measures

The mean length of the fascicles at rest was 59±10 and 37±10 mm for gastrocnemius and soleus, respectively. Both gastrocnemius and soleus MTUs lengthened significantly throughout the whole landing task (Fig. 6A). A greater lengthening was observed for the soleus MTU than for the gastrocnemius MTU during phase 3 (5.5 versus 4.0 mm, respectively, $P\leq0.01$). By contrast, a larger strain was observed in gastrocnemius fascicles than in the soleus ($P\leq0.01$ in all three phases), despite parallel changes in all phases (shortening–lengthening–lengthening). The highest lengthening velocities of gastrocnemius and soleus MTUs were reached during phase 2 (539±155 and 545±145 mm s^{-1}, respectively) (Fig. 7A). Unlike MTUs, gastrocnemius and soleus fascicles shortened significantly in phase 1 and lengthened during the rest of the landing (Table 1, Fig. 6B). Gastrocnemius fascicles lengthened at a faster velocity than soleus fascicles (peak velocities: 214±50 and 132±32 mm s^{-1}, respectively, Fig. 6B). Peak fascicle velocity occurred later than peak MTU velocity. The Achilles tendon and all elastic elements lengthened between t_0 and t_1 as well as between t_1 and t_2. In the last phase (force decay, between t_2 and t_3), Achilles tendon length did not change significantly, whereas elastic elements shortened. Achilles tendon lengthening during phase 2 (i.e. when negative power production was highest) was relatively lower (2.9%) than that of the elastic elements as a whole (3.6%) (Table 1, Fig. 6C,D).

Adding mass to the subjects resulted in larger gastrocnemius and soleus MTU lengthening during phases 2 and 3, when GRF and ankle moment were higher (Fig. 8A,B). For the soleus MTU, a slight lengthening was also observed in phase 1, at low GRF and ankle moment levels. Length change of the Achilles tendon was significantly greater in phase 2 but not in phases 1 and 3 (Fig. 8E,F), whereas elastic element length change differed significantly between mass conditions in phases 1 and 2. Contrarily, gastrocnemius and soleus muscle fascicle lengths did not differ significantly between experiments with body mass and added mass (Fig. 8C,D).

DISCUSSION

Our results support the hypothesis of a buffering function of elastic structures enabled by a decoupling mechanism between the behaviour of the MTU, muscle fascicles and elastic structures during energy dissipation. These findings are consistent with animal studies describing the buffering and shock-absorbing function of the tendon (Konow et al., 2012), and expand on previous studies of human triceps surae behaviour during movements requiring limited energy dissipation (countermovement and stair descent). Hence, in a pure energy dissipation task as performed in the present study, stretching of elastic elements accommodates the initial lengthening of the MTU but, in contrast to other decelerating movements, energy is then quickly dissipated via early and active fascicle lengthening in both the gastrocnemius and soleus. A late recoil of combined elastic elements, but not the Achilles tendon solely, supports our hypothesis of distinct strain patterns of the two structures during energy dissipation.

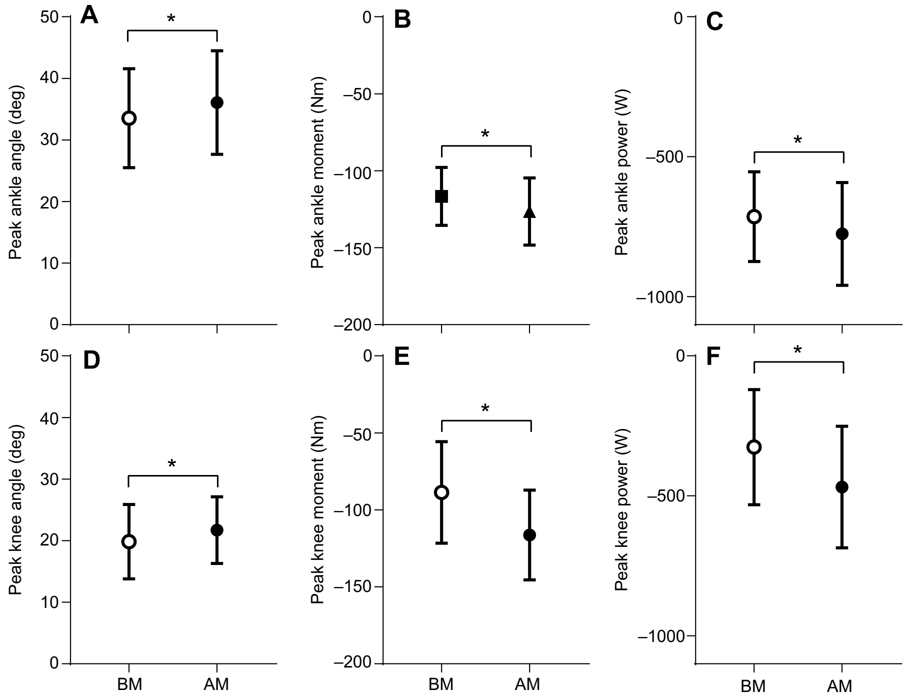

Fig. 3. Comparison of peak values of ankle and knee angle, moment and power between trials with body mass and 20% added mass during energy absorption. Data are means±s.d. *$P<0.05$ when compared with the other condition. BM, body mass; AM, added mass.

The role of elastic structures in energy dissipation

At the onset of negative power, when the joint moment increased slightly, ankle dorsiflexion caused a quasi-isometric followed by a small lengthening of gastrocnemius and soleus MTUs. A concomitant stretch of the tendon and combined elastic elements was observed, whereas gastrocnemius and soleus fascicles shortened (Fig. 6). Energy is thus stored in elastic elements – not dissipated – at this stage and a counteracting plantar flexion moment is generated. In phase 2, characterised by the greatest increase in force, ankle dorsiflexion led to further lengthening of the MTUs. Concurrently, tendon and combined elastic elements lengthened, although to a different extent. At the increased magnitude and velocity of MTU stretch, fascicles started to actively lengthen and dissipate energy. Whereas gastrocnemius muscle activity declined, the activity of soleus peaked in this phase. However, the elongation of elastic elements enabled both gastrocnemius and soleus fascicles to lengthen at a slower rate than their respective MTUs (Fig. 7). Hence, in addition to limiting the length of active fascicles, the uncoupling between fascicles and MTU behaviour enables a delayed, slower contraction of muscle fibres. In animal studies, the mitigation of fascicle lengthening velocity has been linked to a reduction in power input to the fascicles (Konow and Roberts, 2015). We could not apply the same invasive measurements in human subjects, but power input to fascicles was likely limited here, via the buffering action of elastic elements. In support of this assumption, the delay in peak fascicle velocity during this phase enabled peak fascicle lengthening to be uncoupled from MTU stretch (Fig. 7). These findings, and the link between fascicle strain magnitude (e.g. Guilhem et al., 2016) or velocity and muscle damage, are congruent with a protective mechanism against damage. During force decay (phase 3), MTUs lengthened at a much slower rate (about 9% of peak velocity). Elastic elements shortened, whereas the length of the Achilles tendon alone did not

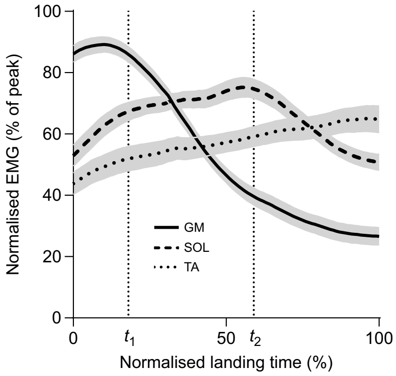

Fig. 4. Electromyographic (EMG) activity of gastrocnemius medialis (GM), soleus (SOL) and tibialis anterior (TA) during energy absorption in the body-mass condition. Data are means±s.e.m. Time series are normalised to 101 points. Values were normalised to peak values during the stepping-down task. Vertical dotted lines represent the time points of the highest rate of GRF development (t_1) and peak GRF (t_2).

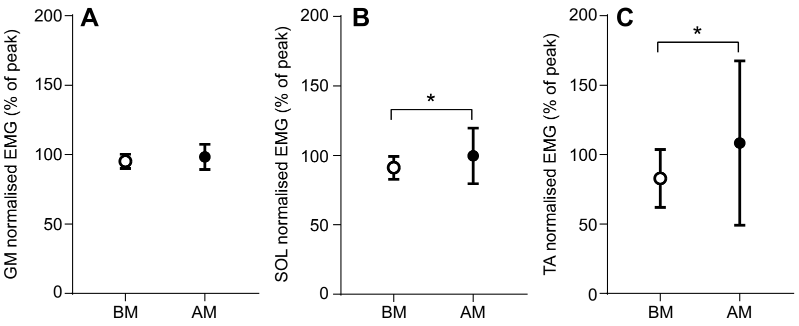

Fig. 5. Comparison of peak EMG activity occurring during energy absorption, with and without added mass. Data are means±s.d. Data from GM (A), SOL (B) and TA (C) muscles are normalised to peak values obtained during the entire stepping-down task. *$P<0.05$ when compared with the other condition.

change. At the end position of the task, whereas no more negative power was produced at the ankle, the Achilles tendon was still strained by 6%, close to the maximal value recorded during the task. The different behaviour between elastic elements and the Achilles tendon highlights the importance of other elastic structures in the triceps surae MTU during energy dissipation. Besides the unknown mechanical properties of the proximal tendon, recent studies (Raiteri et al., 2016; Tilp et al., 2012) have shown the complex role of aponeuroses. Unlike tendons, the elasticity of aponeuroses does not depend on force alone and seems geared by the active state of muscles via changes in the orientation of their fibres. Changes in the pennation angle of fibres are associated with the muscle's architecture gear ratio, whereby changes in fibre length are partly dissociated from muscle – and aponeuroses – strain patterns (Azizi et al., 2008). Although not measured in the present study, the gear ratio is reportedly more important during lengthening than shortening contractions (Azizi and Roberts, 2014) and may explain some of the differences seen between combined elastic elements and the Achilles tendon alone. Additionally, spring-like elements located in muscles (actin–myosin cross-bridges, titin and connective tissue) can store elastic energy during contraction (for a review, see Roberts, 2016). The relative contribution of these elements, in series and in parallel with the tendon, is yet unknown and future studies should establish its functional significance during decelerating movements. However, it should be noted that this difference might partly be explained by methodological reasons, owing to the different techniques and assumptions related to length measurements of these tissues. Whereas elastic element lengths were calculated based on changes in MTU length and muscle architecture, Achilles tendon length was measured directly, with the different possible sources of error such as the assumption of a 2D conformation. Regardless of these considerations, the recoil or

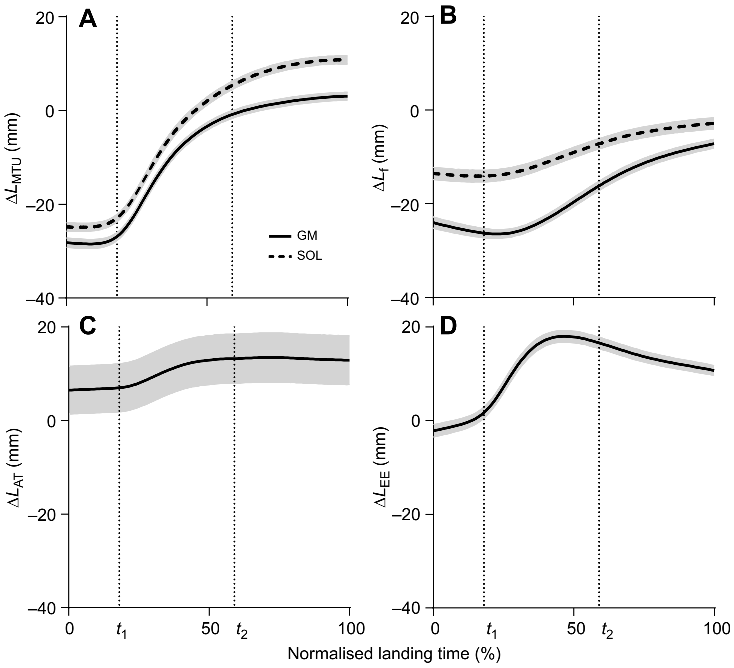

Fig. 6. Instantaneous length changes of muscle-tendon unit (L_{MTU}), fascicles (L_{f}), Achilles tendon (L_{AT}) and elastic elements (L_{EE}) in the unloaded condition during energy absorption. Data are means±s.e.m. Time series are normalised to 101 points. Data are displayed as difference to resting lengths measured when lying prone in the anatomical position. Vertical dotted lines represent the time points of the highest rate of GRF development (t_1) and peak GRF (t_2).

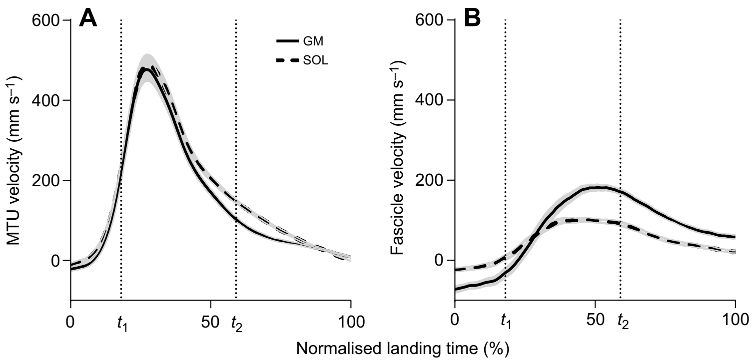

Fig. 7. Instantaneous MTU and fascicle velocity of GM and SOL during energy absorption. Data are means±s.e.m. Time series are normalised to 101 points. Vertical dotted lines represent the time points of the highest rate of GRF development (t_1) and peak GRF (t_2).

isometric behaviour of elastic structures during MTU lengthening drove further fascicle lengthening in phase 3. The occurrence of active fascicle lengthening during the end of the task indicates that energy was being dissipated in phase 3, despite the shortening of the MTU.

The near-constant fascicle lengthening and energy dissipation occurring during a pure energy dissipation task contrasts with previous observations from tasks requiring an acceleration of the body after ground contact. During drop jumps, for instance, gastrocnemius fascicles were found to shorten throughout the contact phase, presumably to generate and retain the elastic energy used during push-off (Ishikawa et al., 2005). Closer to our experimental model, Spanjaard et al. (2007) showed a lengthening of gastrocnemius fascicles during the single-support phase of stair descent. However, differences in the timing of changes in EMG activity and fascicle length distinguish stair descent from the energy dissipation task of the present protocol.

Contrary to our findings, fascicles were found to shorten during stair descent from touch down to nearly the end of the negative power phase. Subsequent lengthening mainly occurred when no power was being produced at the ankle joint, during the single-support phase. The relatively large fascicle shortening and the delayed fascicle lengthening during stair descent indicates that energy is dissipated more slowly and may be recycled to perform the subsequent single-support phase. This may help to regulate the optimal tension within the MTU, favouring motor control more than energy dissipation in this task. This pattern is less marked in the present task, where the sole requirement is rapid and effective energy dissipation. Albeit hypothetical, differences between step landing and stair descent tasks may reflect a regulative mechanism whereby energy in excess of that required to maintain the upright position is either dissipated or recycled towards acceleration of the centre of mass.

Effect of added mass

As expected, equipping the subjects with weighted vests increased GRF and joint moment. The increase in EMG activity of the soleus muscle, but not in the gastrocnemius, with additional load may be explained by the different insertions of the two muscles (monoarticular versus biarticular). In this condition, the greater ankle dorsiflexion caused a larger stretch of both MTUs, whereas lengthening of the biarticular gastrocnemius MTU was partly offset by the increased knee flexion. An increased co-activation of the tibialis anterior muscle probably reflected the higher torque produced by the soleus muscle. Elastic structures acted to absorb the additional work at the ankle, as illustrated by a greater stretch of the tendon and all elastic elements during phase 2 (Fig. 7H,K). Consequently, fascicle lengthening remained unchanged in the added-mass condition. Albeit speculative, the preserved fascicle strain in spite of higher negative work at the ankle may prevent strain-induced damage. Furthermore, limiting fascicle strain may prevent the muscle from operating over less advantageous regions of the force–length relationship (Katz, 1939). During phase 3, the greater lengthening of soleus and gastrocnemius MTUs caused by the added mass was not reflected by any significant difference in tendon, elastic elements or fascicle behaviour. This discrepancy may be ascribed to the larger variability of ultrasound-based measurements and the relatively small changes observed during this phase.

Overall, the increased stretch of tendon and elastic elements with added mass underlines the important role of these structures as a mechanism to control different demands of energy dissipation during human movement. This hypothesis is congruous with an earlier study examining the effects of increased demand for energy dissipation during stair descent by modulating step height (Spanjaard et al., 2008). Similarly to the added-mass condition of

Table 1. Mean length changes relative to the length at t_0 of elastic elements, Achilles tendon, and gastrocnemius medialis and soleus muscle–tendon unit and fascicles at selected time points in the unloaded condition

	ΔL_{MTU} (mm)		ΔL_f (mm)			
Time point	GM	SOL	GM	SOL	ΔL_{AT} (mm)	ΔL_{EE} (mm)
t_1	1.3±2.1*	1.9±2.1*	−2.3±2.2*	−0.5±1.2*	0.5±1.0*	3.9±2.6*
t_2	27.4±7.4*	30.2±7.3*	7.6±6.9*	6.3±3.5*	6.7±4.4*	18.7±7.9*
t_3	31.3±7.2*	35.6±7.2*	16.8±6.6*	10.6±3.2*	6.4±4.2	12.9±7.4*

Data are means±s.d. L_{MTU}, muscle–tendon unit length; L_f, fascicle length; L_{AT}, Achilles tendon length; L_{EE}, elastic element length; GM, gastrocnemius medialis; SOL, soleus. t_0, onset of negative ankle power; t_1, highest rate of ground reaction force (GRF) development; t_2, peak GRF; t_3, end of negative ankle power. *$P<0.05$ when compared with the preceding event.

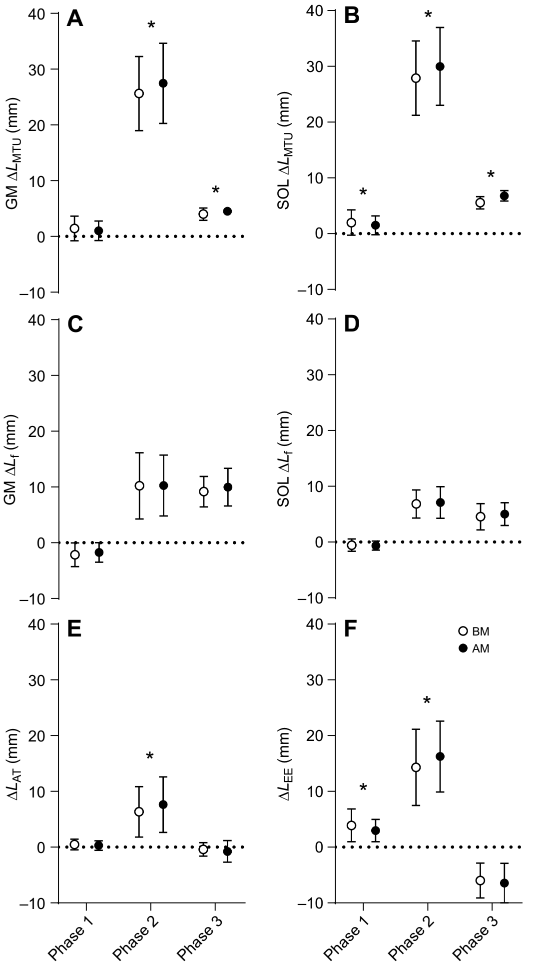

Fig. 8. Length changes of components of the MTU during the landing task, with and without added mass. Data are means±s.d. Sub-phases are based on GRF: near-constant force (phase 1), force rise (phase 2) and force decay (phase 3). $^*P<0.05$ when compared with the other condition.

our study, an increase in step height led to a greater stretch of elastic elements (Spanjaard et al., 2008). However, contrary to our findings, a higher demand for energy dissipation during stair descent caused an increase in fascicle shortening. In line with Spanjaard et al. (2008) and similar observations in animals by Konow and Roberts (2015), increasing body mass would be expected to induce larger fascicle shortening at the onset of the present task. Indeed, an initial shortening of fascicles allows a greater subsequent lengthening and may enable dissipation of more energy. The lack of change in fascicular behaviour with added mass may be ascribed to a different strategy of energy dissipation. As evidenced by the greater EMG activity of the soleus and the longer duration of fascicle lengthening, additional energy in the loaded condition was mainly dissipated via longer, more forceful eccentric contractions in the present task. This further suggests the central role of muscle activation in regulating the rate of energy dissipation according to the task constraints (i.e. complete deceleration or reacceleration of the centre of mass).

Conclusion

During a task requiring energy dissipation, elastic structures of the human triceps surae buffer mechanical energy in a similar manner to that described in drop landings of animals. However, different patterns of muscle activity and fascicle lengthening found between this task and other movements involving eccentric actions suggest that this mechanism can be modulated differently, depending on the movement characteristics. The temporary storage of elastic energy is commensurate with loading constraints to limit the maximal strain and lengthening velocity of muscle fascicles. Additional energy is absorbed during a longer phase of energy absorption and seems to be dissipated via higher force production of the soleus and longer-lasting eccentric contractions. Under the present experimental conditions, the behaviour of the Achilles tendon appeared similar but not identical to that of all elastic elements, implying that the function of the two structures cannot be studied interchangeably. Finally, our results show that the pattern of fascicle lengthening during active energy dissipation is similar in the biarticular gastrocnemius and in the monoarticular soleus. However, the two muscles may contribute differently to an increased demand for energy dissipation.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.W., K.A., N.J.C., J.B.-M., O.R.S.; Methodology: A.W., K.A., N.J.C., O.R.S.; Formal analysis: A.W., R.M.; Investigation: A.W., R.M.; Data curation: A.W., R.M.; Writing - original draft: A.W., O.R.S.; Writing - review & editing: A.W., K.A., N.J.C., R.M., J.B.-M., O.R.S.; Visualization: A.W.; Supervision: O.R.S.; Project administration: A.W., O.R.S.

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References

Azizi, E. and Roberts, T. J. (2014). Geared up to stretch: pennate muscle behavior during active lengthening. *J. Exp. Biol.* **217**, 376-381.

Azizi, E., Brainerd, E. L. and Roberts, T. J. (2008). Variable gearing in pennate muscles. *Proc. Natl. Acad. Sci. USA* **105**, 1745-1750.

Bell, A. L., Brand, R. A. and Pedersen, D. R. (1989). Prediction of hip joint centre location from external landmarks. *Hum. Mov. Sci.* **8**, 3-16.

Cronin, N. J., Carty, C. P., Barrett, R. S. and Lichtwark, G. (2011). Automatic tracking of medial gastrocnemius fascicle length during human locomotion. *J. Appl. Physiol.* **111**, 1491-1496.

Cronin, N. J., Avela, J., Finni, T. and Peltonen, J. (2013). Differences in contractile behaviour between the soleus and medial gastrocnemius muscles during human walking. *J. Exp. Biol.* **216**, 909-914.

Farris, D. J. and Lichtwark, G. A. (2016). UltraTrack: software for semi-automated tracking of muscle fascicles in sequences of B-mode ultrasound images. *Comput. Methods Programs Biomed.* **128**, 111-118.

Farris, D. J., Lichtwark, G. A., Brown, N. A. T. and Cresswell, A. G. (2016). The role of human ankle plantar flexor muscle–tendon interaction and architecture in maximal vertical jumping examined in vivo. *J. Exp. Biol.* **219**, 528.

Fukunaga, T., Kubo, K., Kawakami, Y., Fukashiro, S., Kanehisa, H. and Maganaris, C. N. (2001). In vivo behaviour of human muscle tendon during walking. *Proc. R. Soc. Lond. B. Biol. Sci.* **268**, 229-233.

Griffiths, R. I. (1991). Shortening of muscle fibres during stretch of the active cat medial gastrocnemius muscle: the role of tendon compliance. *J. Physiol.* **436**, 219-236.

Guilhem, G., Doguet, V., Hauraix, H., Lacourpaille, L., Jubeau, M., Nordez, A. and Dorel, S. (2016). Muscle force loss and soreness subsequent to maximal eccentric contractions depend on the amount of fascicle strain in vivo. *Acta Physiol.* **217**, 152-163.

Hawkins, D. and Hull, M. L. (1990). A method for determining lower extremity muscle-tendon lengths during flexion/extension movements. *J. Biomech.* **23**, 487-494.

Hermens, H. J., Freriks, B., Disselhorst-Klug, C. and Rau, G. (2000). Development of recommendations for SEMG sensors and sensor placement procedures. *J. Electromyogr. Kinesiol.* **10**, 361-374.

Ishikawa, M., Niemelä, E. and Komi, P. V. (2005). Interaction between fascicle and tendinous tissues in short-contact stretch-shortening cycle exercise with varying eccentric intensities. *J. Appl. Physiol.* **99**, 217-223.

Katz, B. (1939). The relation between force and speed in muscular contraction. *J. Physiol.* **96**, 45-64.

Kawakami, Y., Muraoka, T., Ito, S., Kanehisa, H. and Fukunaga, T. (2002). In vivo muscle fibre behaviour during counter-movement exercise in humans reveals a significant role for tendon elasticity. *J. Physiol.* **540**, 635-646.

Konow, N. and Roberts, T. J. (2015). The series elastic shock absorber: tendon elasticity modulates energy dissipation by muscle during burst deceleration. *Proc. R. Soc. Lond. B. Biol. Sci.* **282**, 20142800**.**

Konow, N., Azizi, E. and Roberts, T. J. (2012). Muscle power attenuation by tendon during energy dissipation. *Proc. R. Soc. Lond. B. Biol. Sci.* **279**, 1108.

Kristianslund, E., Krosshaug, T. and van den Bogert, A. J. (2012). Effect of low pass filtering on joint moments from inverse dynamics: implications for injury prevention. *J. Biomech.* **45**, 666-671.

Kurokawa, S., Fukunaga, T. and Fukashiro, S. (2001). Behavior of fascicles and tendinous structures of human gastrocnemius during vertical jumping. *J. Appl. Physiol.* **90**, 1349-1358.

Lichtwark, G. A. and Wilson, A. M. (2005). In vivo mechanical properties of the human Achilles tendon during one-legged hopping. *J. Exp. Biol.* **208**, 4715-4725.

Lichtwark, G. A. and Wilson, A. M. (2006). Interactions between the human gastrocnemius muscle and the Achilles tendon during incline, level and decline locomotion. *J. Exp. Biol.* **209**, 4379-4388.

Maharaj, J. N., Cresswell, A. G. and Lichtwark, G. A. (2016). The mechanical function of the tibialis posterior muscle and its tendon during locomotion. *J. Biomech.* **49**, 3238-3243.

Raiteri, B. J., Cresswell, A. G. and Lichtwark, G. A. (2016). Three-dimensional geometrical changes of the human tibialis anterior muscle and its central aponeurosis measured with three-dimensional ultrasound during isometric contractions. *PeerJ* **4**, e2260.

Roberts, T. J. (2016). Contribution of elastic tissues to the mechanics and energetics of muscle function during movement. *J. Exp. Biol.* **219**, 266-275.

Roberts, T. J. and Azizi, E. (2010). The series-elastic shock absorber: tendons attenuate muscle power during eccentric actions. *J. Appl. Physiol.* **109**, 396-404.

Roberts, T. J. and Azizi, E. (2011). Flexible mechanisms: the diverse roles of biological springs in vertebrate movement. *J. Exp. Biol.* **214**, 353-361.

Spanjaard, M., Reeves, N. D., van Dieën, J. H., Baltzopoulos, V. and Maganaris, C. N. (2007). Gastrocnemius muscle fascicle behavior during stair negotiation in humans. *J. Appl. Physiol.* **102**, 1618-1623.

Spanjaard, M., Reeves, N. D., van Dieën, J. H., Baltzopoulos, V. and Maganaris, C. N. (2008). Lower-limb biomechanics during stair descent: influence of step-height and body mass. *J. Exp. Biol.* **211**, 1368-1375.

Tilp, M., Steib, S. and Herzog, W. (2012). Length changes of human tibialis anterior central aponeurosis during passive movements and isometric, concentric, and eccentric contractions. *Eur. J. Appl. Physiol.* **112**, 1485-1494.

Article 2

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Effect of Training-Induced Changes in Achilles Tendon Stiffness on Muscle–Tendon Behavior During Landing

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During rapid deceleration of the body, tendons buffer part of the elongation of the muscle–tendon unit (MTU), enabling safe energy dissipation via eccentric muscle contraction. Yet, the influence of changes in tendon stiffness within the physiological range upon these lengthening contractions is unknown. This study aimed to examine the effect of training-induced stiffening of the Achilles tendon on triceps surae muscle–tendon behavior during a landing task. Twenty-one male subjects were assigned to either a 10-week resistance-training program consisting of single-leg isometric plantarflexion ($n = 11$) or to a non-training control group ($n = 10$). Before and after the training period, plantarflexion force, peak Achilles tendon strain and stiffness were measured during isometric contractions, using a combination of dynamometry, ultrasound and kinematics data. Additionally, testing included a step-landing task, during which joint mechanics and lengths of gastrocnemius and soleus fascicles, Achilles tendon, and MTU were determined using synchronized ultrasound, kinematics and kinetics data collection. After training, plantarflexion strength and Achilles tendon stiffness increased (15 and 18%, respectively), and tendon strain during landing remained similar. Likewise, lengthening and negative work produced by the gastrocnemius MTU did not change detectably. However, in the training group, gastrocnemius fascicle length was offset (8%) to a longer length at touch down and, surprisingly, fascicle lengthening and velocity were reduced by 27 and 21%, respectively. These changes were not observed for soleus fascicles when accounting for variation in task execution between tests. These results indicate that a training-induced increase in tendon stiffness does not noticeably affect the buffering action of the tendon when the MTU is rapidly stretched. Reductions in gastrocnemius fascicle lengthening and lengthening velocity during landing occurred independently from tendon strain. Future studies are required to provide insight into the mechanisms underpinning these observations and their influence on energy dissipation.

Keywords: Achilles tendon, energy absorption, mechanical buffer, stiffness, tendon properties, energy dissipation

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INTRODUCTION

Elastic properties of tendons are inextricably linked to the mechanical output of the muscle to which they are attached. The function of elastic tissue and energy storage includes but is not limited to the conservation of energy or the power amplification observed during locomotor tasks (Roberts and Azizi, 2011). As shown in animals (Roberts and Azizi, 2010; Roberts and Konow, 2013) and in humans (Werkhausen et al., 2017), tendons also act as mechanical buffers to accommodate rapid stretches of the muscle–tendon unit (MTU) and thus contribute to mechanical energy dissipation via lengthening contractions. The buffering function of the tendon provides a controlled means for the MTU to absorb energy, and is associated with a mechanism to protect muscle fascicles against damage caused by rapid and excessive strain (for review see Roberts and Konow, 2013). Thus, during a task where power attenuation is required, the tendon mechanical properties affect the active lengthening of muscle fibers and their ability to dissipate energy. It follows that changes in tendon properties may affect muscle–tendon interaction and energy dissipation in the MTU.

There is a consensus among studies regarding tendon's adaptability to mechanical loading (Wiesinger et al., 2015). In humans, several studies have demonstrated a stiffening of the Achilles tendon after various types of resistance training (e.g., Kubo et al., 2002; Arampatzis et al., 2007). Additionally, tendinous adaptations seem to occur in parallel with muscular adaptations (Miller et al., 2005; Seynnes et al., 2009), suggesting the influence of the former on work production during locomotor activities.

Modeling studies have demonstrated the theoretical link between locomotion efficiency, tendon stiffness and muscle architecture. Simulations using Hill-type muscle models have shown that during walking and running, contractile efficiency is maximized at optimal combinations of tendon compliance and muscle fascicle length (Lichtwark and Wilson, 2007, 2008). Accordingly, a recent study reported that muscle–tendon behavior during a stretch-shortening exercise was altered after a training intervention that increased Achilles tendon stiffness (Hirayama et al., 2017). Hirayama et al. (2017) interpreted these findings as an optimisation of the muscle–tendon behavior to produce power, via a decrease in fascicle shortening velocity and an increase in tendon shortening velocity. A similar relation between changes in tendon compliance and muscle contractile behavior may apply to lengthening contractions in energy dissipation tasks, but this question has not been investigated to date.

Hence, the aim of this study was to investigate the effect of a resistance training-induced increase in Achilles tendon stiffness on the behavior of the triceps surae MTU during a task requiring energy dissipation. We predicted that training-induced stiffening of the Achilles tendon would reduce tendon strain during the landing task. This decrease was in turn expected to reduce the buffering action of the tendon and increase the magnitude and velocity of fascicle lengthening.

MATERIALS AND METHODS

Experimental Protocol and Participants

Twenty-one recreationally active volunteers provided written informed consent to participate in the present study, which was approved by the ethical committee of the Norwegian School of Sport Sciences. *A priori* power calculations based on a previously observed increase in Achilles tendon stiffness with isometric training (Arampatzis et al., 2007) suggested a power of 0.96 with a sample size of $n = 8$. Eleven subjects were assigned to the training group (height 174 ± 9 cm, body mass 70 ± 9 and 69 ± 9 kg before and after training, respectively, age 26 ± 4 years, five men and six women). Exclusion criteria were musculoskeletal disorders preventing the possibility to perform resistance training, and regular strength training of the plantar flexor muscles prior to the study. The subjects in the training group underwent a regimen of explosive, isometric plantarflexions for 10 weeks, while the remaining ten subjects (height 178 ± 8 cm, body mass 73 ± 8 kg before and after training, age 30 ± 3 years, six men and four women) were assigned to the control group. Control subjects did not engage in any resistance training for the plantar flexors and were instructed not to change their daily activities during the course of the study. All participants went through the same protocol before and after the training period where (1) anthropometrics and mechanical properties of the triceps surae MTU were measured, and (2) muscle–tendon behavior during an energy dissipation task was examined by use of ultrasonography, kinematics and kinetics analyses. Before testing subjects performed a warm-up exercise consisting of 5-min running on a treadmill at self-selected speed. For the energy dissipation task, subjects were asked to perform single-leg drop landings from a height of 15 cm. This height was chosen to allow landing with minimal knee flexion. All measurements were performed on the right leg.

Exercise Program

The training group performed standing isometric unilateral plantarflexions three times per week for 10 weeks. Each training session started with a 5-min warm up on a cycle ergometer, followed by four sets of ten explosive contractions (1 s loading, 5 s rest). Explosive contractions with a short time under tension were used in an attempt to minimize strength gains and muscle hypertrophy (Cormie et al., 2010; Balshaw et al., 2016) while eliciting a stiffening of the Achilles tendon. For the training task, subjects stood on one leg in an adjustable and rigid custom-built rig with their ankle and knee joints in anatomical position, facing a wall. The position of horizontal shoulder arms attached to the wall was adjusted to the shoulder height of each individual. A taut cable that ran vertically between the shoulder arms and the ground prevented upward displacement of the subjects' shoulders, enabling isometric plantar flexion contractions. Strength measurements used to monitor and adjust training load were obtained from a strain gauge placed in series with the vertical cable. Subjects were instructed to reach the target force as fast as possible. The contraction intensity was set to 80% of maximum force and was adjusted during the first training session of every week. Pilot testing indicated that this force level was the highest that subjects could reach reliably across four sets of 10 repetitions. Visual feedback of instantaneous force was provided to the subjects to enable them to match the target force during training and to visualize maximal force during testing.

Properties of the Muscle–Tendon Unit

Muscle Architecture

Ultrasound images of resting muscle architecture were taken from the mid-belly of the muscle (i.e., mid-length between the popliteal fossa and the gastrocnemius myotendinous junction and along the mediolateral width) when the subjects were lying prone with the hip, knee, and ankle joints in anatomical position (HL9.0/60/128Z-2, LS 128 Telemed, Vilnius, Lithuania). Fascicle length, pennation angle and thickness of gastrocnemius medialis were measured offline with software for image analysis (ImageJ, National Institutes of Health, Bethesda, MD, United States). Fascicle length was measured as a straight line aligned with visible portions of fascicles, between the superficial and deep aponeuroses. A linear extrapolation was necessary in the few cases when the fascicles did not fit within the 60 mm field of view. Pennation angle was defined as the angle between the segmented fascicle and the orientation of the deep aponeurosis. Muscle thickness was calculated as the average of the shortest distances between the two aponeuroses, measured at 25, 50, and 75% of the width of the field of view.

Maximal Voluntary Torque

Subjects lay prone on a dynamometer (IsoMed 2000 D. & R. Ferstl GmbH, Hemau, Germany) with hip, knee, and ankle joints firmly fixed at anatomical positions. Stiff pads and straps were adjusted to restrict the movement of the trunk and knee joint. The axis of the dynamometer was carefully aligned with the rotation axis of the ankle joint, and foot straps, knee and shoulder pads were adjusted to minimize movement. As a specific warm up, subjects performed at least five submaximal contractions of the plantar flexors. The maximal plantarflexion torque was determined as the highest of at least two maximal voluntary contractions.

Achilles Tendon Mechanical Properties

In the same position, ultrasound scans (80 Hz) of the gastrocnemius myotendinous junction, plantarflexion torque (600 Hz) and kinematic marker trajectories (120 Hz) were recorded simultaneously during ramp contractions to estimate tendon stiffness. The ultrasound transducer was fixed with adhesive tape over the myotendinous junction of the gastrocnemius. A gel pad was positioned between the skin and the transducer to allow consistent scanning when the muscle was bulging. Four motion analysis cameras (Qualisys, Gothenburg, Sweden) captured the positions of three reflective markers attached to the ultrasound probe and one marker on the calcaneus over the tendon insertion. A trigger signal from the ultrasound system synchronized all measurements. Subjects were provided with visual feedback and instructed to exert a torque at a loading rate of 100 N m s^{-1} up to 90% of the individual maximal torque. Ramp contractions at constant loading rate were performed before trials were recorded, to familiarize the subjects with the task and to ensure preconditioning of the tendon (Maganaris, 2003b).

Marker trajectories were filtered with a second order bidirectional low-pass Butterworth filter with a cut-off frequency of 15 Hz. The position of the gastrocnemius myotendinous junction was tracked offline semi-automatically by following the closest visible fascicle insertion (Tracker 4.95[1]). The fascicle insertion was chosen in a region 1–3 cm proximal to the actual muscle–tendon junction. The distance between the tracked features and the muscle–tendon junction was constant in all the video scans of each subject. Ultrasound data were filtered with a second order bidirectional low-pass Butterworth filter with a cut-off frequency of 6 Hz. Prior calibration established the position of the ultrasound image relative to the kinematic markers on the cast used to hold the ultrasound transducer. This enabled calculation of the position of the myotendinous junction in the laboratory coordinates system. Thus, Achilles tendon length was calculated as the distance between the myotendinous junction and the calcaneus marker during the contraction (Gerus et al., 2011). Plantarflexion torque data was filtered, similarly to the kinematic data, and the Achilles tendon force was estimated by dividing the torque by the internal moment arm, measured externally with a tape measure as the mean perpendicular distance from the tendon to the midpoint between the medial and lateral malleolus. For further analysis, 91% of the calculated tendon force was used to represent the proportion of total ankle moment attributable to the triceps surae (Dick et al., 2016). Using kinematics, the torque was corrected to account for inevitable ankle joint rotation (Arampatzis et al., 2005) and gravitational forces (Karamanidis et al., 2005). Subsequently, the tendon force-elongation plots of three out of five trials, where the trials with the highest and lowest stiffness were excluded, were averaged and fitted with a third order polynomial. Tendon stiffness was calculated for every subject as the slope of the fitted force-elongation curve between 50 and 80% of the maximum individual force level, and maximum tendon strain was measured at the maximum common force of pre- and post-tests for every subject.

Mechanics of the Landing Task

Joint Mechanics

Ankle and knee joint mechanics were measured from the right leg during single-leg landings. Twenty retroreflective markers were attached to the skin and captured by at least 12 cameras operating at 300 Hz. Motion capture was synchronized with force plates recording at 1500 Hz (Force-Link, Motek, Netherlands). A modified Cleveland Clinics marker set (left and right anterior and posterior iliac spine; right medial and lateral epicondyles; right medial and lateral malleoli; posterior calcaneus and first, second, and fifth metatarsal; two clusters of four markers to track the right thigh and shank segment, respectively) was used to calculate ankle and knee joint angles, moments and powers (Visual 3D, C-Motion, Germantown, MD, United States). For the purpose of the analysis, the landing phase was defined as the phase of negative ankle power production. Ground reaction force (GRF) and trajectory data were filtered at 15 Hz

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[1] physlets.org/tracker/

using a bidirectional second order low-pass Butterworth filter. Gastrocnemius and soleus MTU lengths were estimated from ankle and knee joint angles and shank length data (Hawkins and Hull, 1990). Shank length was measured externally, from the lateral malleolus to the lateral epicondyle.

Tendon Mechanics

Achilles tendon length during landing was estimated using the same procedure as that described above for the measurement of tendon stiffness, with the same frame rate of 80 Hz. The instantaneous Achilles tendon moment arm was calculated as the shortest perpendicular distance from the tendon to the ankle joint center defined as the midpoint of the malleoli markers (Obst et al., 2017). Achilles tendon force was then estimated by dividing ankle joint moment by the moment arm (Fukashiro et al., 1993).

Fascicle Mechanics

Ultrasound videos were recorded in additional trials to examine changes in gastrocnemius and soleus fascicle length. The use of ultrasonography to determine muscle fascicle length has previously been shown to provide satisfactory levels of reliability (Gillett et al., 2013; Kwah et al., 2013). The transducer was positioned over the gastrocnemius muscle belly, so that soleus fascicles were also visible. A transducer cast and self-adhesive tape prevented movement of the transducer. Fascicle lengths and pennation angles were obtained using a semi-automated tracking algorithm (Cronin et al., 2011; Farris and Lichtwark, 2016). Ultrasound images from each group were analyzed in a random order (pre-post) by the same investigator. Fascicle, tendon, and MTU velocities were obtained by differentiating the corresponding length with respect to time.

Data Reduction and Statistical Analysis

Analysis of changes in variables of interest during the landing task was performed over the period of negative power production at the ankle joint, to reflect the duration of energy absorption and dissipation. All time series data were resampled over 101 points and averaged across 4–5 trials per subject. Individual mean time series were subsequently averaged across subjects for each group. A repeated-measures, two-way ANOVA with the factors time of testing (pre- vs. post training) and group (training group vs. control group) was used to test differences in Achilles tendon properties, muscle architecture and muscle–tendon behavior at relevant phases of the landing task. Sidak *post hoc* tests were employed in case of significant main effects or time by group interactions. Pearson correlation coefficients were calculated between the changes in tendon stiffness and changes in tendon strain and fascicle lengthening during landing. Statistical significance was set to $P < 0.05$. Results are presented as mean ± standard deviation in the text and as mean ± standard error of the mean in the line figures to illustrate the precision of the mean.

RESULTS

Muscle–Tendon Properties and Maximal Strength

Table 1 shows the changes in muscle–tendon properties of both groups after the training period. The one repetition maximum of the training group increased from 1670 ± 393 N in week one to 2317 ± 607 N in week 10. Maximal isometric plantarflexion torque increased on average by 15%, with concomitant 5% increases in gastrocnemius pennation angle and muscle thickness, without any change in fascicle length. Achilles tendon stiffness measured before and after training increased on average by 18%, although the strain at the individualized maximal force reached in pre- and post-intervention tests (1683 ± 463 N) did not decrease significantly (mean change: −9%, mean ± SD: 3.4 ± 0.7 and 3.1 ± 0.5%). Individual and mean force-elongation relationship of the Achilles tendon are presented in the Supplementary Figure S1. None of these variables changed significantly in the control group.

Kinematics and Kinetics During the Landing Task

Subjects in both groups executed the landing task in the same way at pre- and post-training, as evidenced by similar landing duration, peak GRF, knee and ankle joint moment and power, and Achilles tendon force (**Table 2**). While control subjects landed in a less plantar flexed position than trained subjects ($P = 0.001$), in the training group the ankle angle at touch down was less plantar flexed after training ($P = 0.026$). While no interaction effect was

TABLE 1 | Plantarflexion strength (torque), resting muscle architecture and Achilles tendon (AT) stiffness in the training group (TG) and the control group (CG) measured in the pre-test (pre) and the post-test (post).

	TG			CG		
	Pre	**Post**	**CI**	**Pre**	**Post**	**CI**
Torque [N m]	172 ± 50	198 ± 51	−11 to −42*	170 ± 51	180 ± 62	7 to −27
L_f GM [mm]	892 ± 133	907 ± 161	42 to −72	842 ± 161	840 ± 104	62 to −59
PA GM [deg]	18.1 ± 1.8	19.0 ± 2.0	0 to −1.7*	18.4 ± 1.1	18.3 ± 1.7	1 to −0.8
Thickness GM [mm]	237 ± 35	250 ± 38	0 to −27*	231 ± 38	235 ± 36	9 to −18
AT stiffness [N mm^{-1}]	397 ± 146	459 ± 147	−14 to −109*	399 ± 193	400 ± 212	48 to −51
AT strain [mm]	4.4 ± 1.1	4.1 ± 0.5	−0.3 to 1.1	4.0 ± 1.5	3.9 ± 1.5	−0.6 to 0.8

*Values are means ± SD; CI, 95% confidence interval of differences; GM, gastrocnemius medialis; SOL, soleus; L_f, length of fascicle; PA, pennation angle; *$P < 0.05$ comparing pre and post-test.*

TABLE 2 | Peak ankle and knee moment and power, Achilles tendon (AT) force, MTU work and landing duration in the training group (TG) and the control group (CG) measured in the pre-test (pre) and the post-test (post).

	TG		CG	
	Pre	**Post**	**Pre**	**Post**
Ankle moment [N m]	−143 ± 34	−141 ± 30	−153 ± 26	−154 ± 34
Ankle power [W]	−976 ± 260	−1012 ± 186	−1027 ± 192	−998 ± 193
Ankle work [J]	−78 ± 25	−70 ± 30	−74 ± 21	−69 ± 13
Knee moment [N m]	90 ± 45	108 ± 42	117 ± 45	112 ± 40
Knee power [W]	−314 ± 203	−455 ± 218	−549 ± 245	−532 ± 312
Knee work [J]	−16 ± 16	−19 ± 13	−27 ± 15	−20 ± 16
AT force [N]	2746 ± 555	2750 ± 518	2848 ± 493	2857 ± 736
MTU work [J]	40 ± 13	39 ± 10	34 ± 8	30 ± 7
Duration [s]	0.205 ± 0.014	0.202 ± 0.013	0.204 ± 0.020	0.202 ± 0.020

Values are means ± SD; AT, Achilles tendon, no statistical differences were observed between pre- and post-test.

found for the ankle angle at touch down, a group effect showed that control subjects landed in a less plantar flexed position than trained subjects ($P = 0.001$). The analysis also showed a main effect for time, with *post hoc* tests indicating a less plantar flexed ankle angle at touchdown after training for the training group ($P = 0.026$) but not for the control group ($P = 0.306$). The excursion of the ankle joint during the task did not change significantly after the intervention, as evidenced by a lack of significant group × time interaction effect ($P = 0.937$). A trend toward a reduced plantarflexion during landing was observed in post-tests (−6% in the training group and −7% in the control group) but did not reach significance ($P = 0.110$). Knee joint angle at touchdown and knee joint excursion were statistically similar between groups and after training (**Figure 1**).

Muscle–Tendon Measures During the Landing Task

The mean changes in length of MTUs and their components (fascicles, Achilles tendon only, and muscle) during landing are presented for each group, before and after training, in **Figure 2**. After an initial isometric phase (≈20% of landing duration), soleus and gastrocnemius MTUs lengthened until the end of the landing period. During the first half of the landing, the Achilles tendon stretched, similarly to the whole MTU and did not lengthen further in the second half. In contrast, muscle fascicles initially shortened ($P < 0.001$), before lengthening throughout the rest of the landing period ($P < 0.001$). In line with the differences in ankle joint angle mentioned above, group differences in length of MTU were observed at touch down. In addition, gastrocnemius – but not soleus – muscle and fascicles lengthened more during landing in the training group than in controls before the intervention (**Table 3**).

At the beginning of the negative power phase, gastrocnemius fascicles were longer for the training group after training ($P = 0.012$) whereas this variable did not change for the control group ($P = 0.930$). Soleus fascicle length at this time point was similar before and after training ($P = 0.685$). Consistent with fascicle length, gastrocnemius pennation angle at touchdown was lower after training ($P = 0.035$), while tendon length did not change ($P = 0.707$).

After the training intervention, the onset of fascicle lengthening occurred later for the training group (+7% of the landing period for the gastrocnemius, $P = 0.002$ and +8% for the soleus, $P = 0.014$, respectively) but not for the control group (+1%, $P = 0.788$ and +3%, $P = 0.614$, respectively). A significant effect of training was also seen on the magnitude of gastrocnemius and soleus fascicle lengthening. *Post hoc* tests showed that fascicle lengthening of both muscles decreased in the training group only (**Table 3** and **Figure 2**). Accordingly, peak shortening and mean shortening velocity of the gastrocnemius fascicles were significantly reduced in the trained group, although this reduction did not reach significance for soleus (**Table 3** and **Figure 3**). To ensure that the observed effects of training on fascicle length and velocity were not due to slight differences in the execution of the task between groups and time points, the same statistical analysis was performed on fascicle length values normalized to instantaneous MTU length. This additional analysis confirmed the differences described in **Table 3** and **Figure 4**. Neither fascicle shortening nor lengthening of MTU and tendon differed significantly after the training period (**Table 3**). No significant correlations were found between the change in stiffness and tendon strain or fascicle lengthening ($r = -0.055$ and $P = 0.814$, $r = -0.373$ and $P = 0.096$).

DISCUSSION

The aim of this study was to examine the effects of a training-induced increase in Achilles tendon stiffness on muscle–tendon mechanics when power attenuation is required, during a drop landing task. Our main hypothesis was that a stiffer tendon would less effectively buffer the lengthening of muscle fascicles against MTU lengthening. Before training, the overall behavior of muscle fascicles and elastic structures during the landing task was consistent with previous observations, showing the buffering function of elastic structures to limit the amplitude and velocity of fascicle lengthening (Roberts and Azizi, 2010;

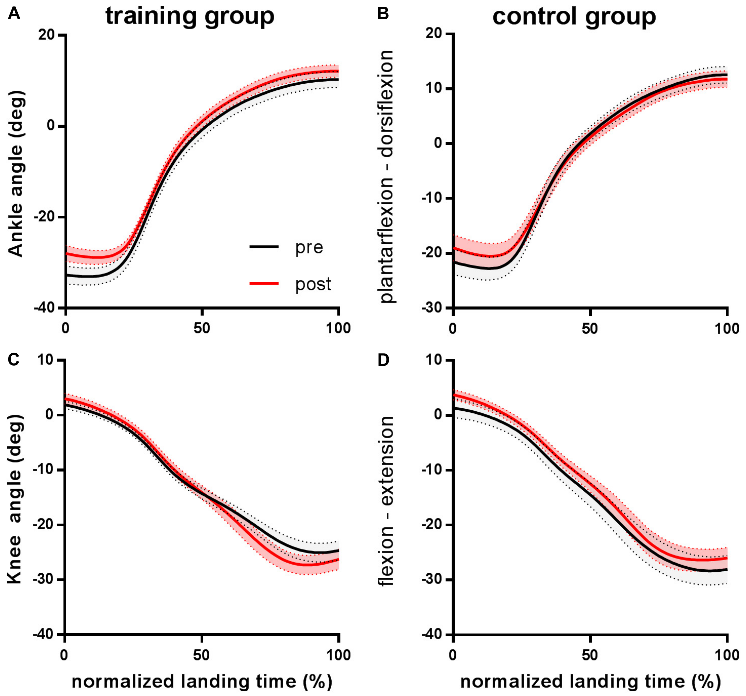

FIGURE 1 | Group mean ankle **(A,B)** and knee joint **(C,D)** angles during energy absorption in the training group and the control group before (pre) and after (post) the training period. Time series are normalized to 101 points. Negative changes in angles correspond to ankle plantarflexion and knee flexion.

Werkhausen et al., 2017). The training intervention increased Achilles tendon stiffness (18%) and muscle strength (15%). Contrary to our predictions, these adaptations did not change the elongation of the Achilles tendon but were accompanied by a reduction in gastrocnemius fascicle lengthening during the negative ankle power phase. Despite reduced fascicle lengthening and unchanged joints moment, the same amount of negative work was done at the ankle and the knee after training. These results support the notion that altered tendon properties can influence the contractile behavior of the triceps surae muscles during a landing task, although the impact of these changes on energy flow remains elusive.

Changes in Muscle–Tendon Properties

Training-induced changes in muscle architecture vary considerably between reports and may depend on training modalities (i.e., contraction type and velocity, (for review see Timmins et al., 2016). Relatively small but significant increases in pennation angle and muscle thickness were measured in the present study. These adaptations are typically associated with additional sarcomeres in parallel and are consistent with the observed increase in plantarflexion strength. However, the fascicle length was not affected by the training protocol.

The 18% increase in tendon stiffness induced by the present protocol is consistent with results from previous interventions based on isometric contractions (Arampatzis et al., 2010; Albracht and Arampatzis, 2013). Although a reduction in strain measured during isometric contractions was expected with tendon stiffening, this variable did not change significantly in the present study, which is nevertheless consistent with some previous reports (e.g., Arampatzis et al., 2007). This apparent discrepancy may be attributable to the lack of sensitivity of tendon strain measurements and the difficulty to assess tendon slack length *in vivo* (Seynnes et al., 2015). Additionally, while we were able to test the Achilles tendon, changes in the mechanical properties of other elastic elements (i.e., aponeuroses, connective tissue, and proximal tendon) could not be assessed, and it is speculated that other collagen-based structures may also have become stiffer as the result of the intervention. These possibilities can unfortunately not be verified here, and we conclude that tendon stiffening was insufficient to reduce Achilles tendon strain significantly.

Landing Kinematics

The more dorsiflexed position at the beginning of the landing in the training group and the same trend in the control

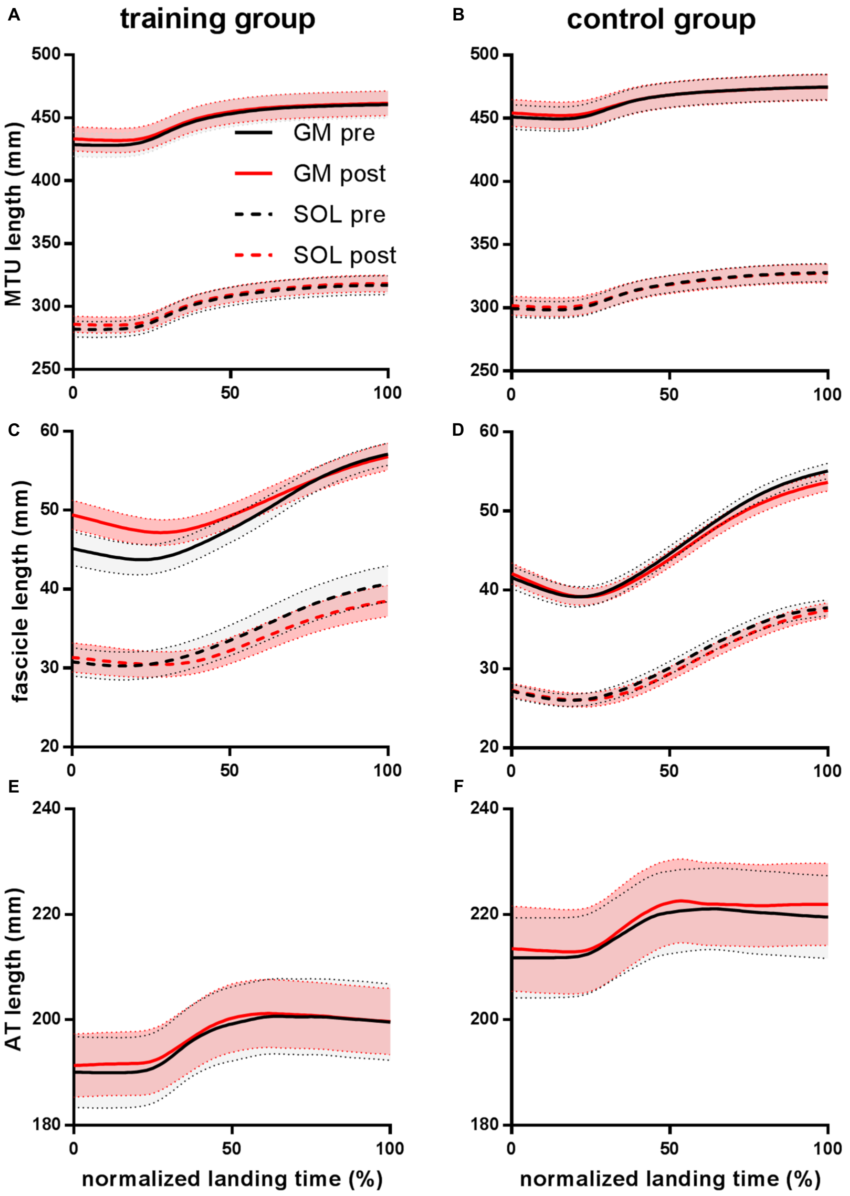

FIGURE 2 | Muscle–tendon unit (MTU) **(A,B)** and fascicle **(C,D)** lengths of gastrocnemius medialis (GM) and soleus (SOL), and Achilles tendon (AT) length **(E,F)**. Time series are normalized to 101 points.

group suggests that a slight change in the kinematic strategy occurred between pre- and post-tests. Despite the practice trials included in our protocol, this trend is possibly attributable to an improvement of the task execution. Alternatively, unaccounted effects of the training intervention (e.g., change in optimal angle of torque production, increase in rate of force development) may have led the trained subjects to land in a less plantarflexed position after training. However, since no

TABLE 3 | Peak length changes and velocities of the muscle–tendon unit (MTU), muscle fascicles, elastic element (EE), Achilles tendon (AT), and muscle length during landing in the training group (TG) and the control group (CG) measured during pre-test (pre) and post-test (post).

		TG			CG		
		Pre	**Post**	**CI**	**Pre**	**Post**	**CI**
Δl Max lengthening [mm]	MTU GM ¤	−32 ± 7	−30 ± 6	-7.1 to 1.4	−25 ± 6	−23 ± 6	−6.9 to 2.0
	MTU SOL	−35 ± 7	−33 ± 5	−6.3 to 2.0	−30 ± 6	−27 ± 6	−6.7 to 2.1
	Fascicles GM ¤	−14 ± 4	−10 ± 2	−6.1 to −1.1*§	−16 ± 3	−15 ± 2	−4.0 to 1.1
	Fascicles SOL	−11 ± 4	−8 ± 2	−4.2 to −0.2*	−12 ± 3	−11 ± 2	−2.4 to 1.8
	AT GM	−12 ± 2	−11 ± 3	−2.3 to 1.0	−10 ± 3	−11 ± 3	−1.0 to 2.6
	Muscle GM	−23 ± 7	−22 ± 6	−5.5 to 3.4	−18 ± 7	−15 ± 7	−7.4 to 1.8
Peak vel [mm s^{-1}]	MTU GM	617 ± 163	560 ± 118	−41 to 155	508 ± 131	451 ± 132	−46 to 160
	MTU SOL	610 ± 151	567 ± 100	−47 to 134	514 ± 115	467 ± 114	−47 to 142
	fascicles GM	134 ± 44	106 ± 26	0 to 56*§	155 ± 24	148 ± 24	−23 to 36
	fascicles SOL	102 ± 26	91 ± 17	−9 to 30	118 ± 32	112 ± 19	−15 to 25
	AT GM	265 ± 36	250 ± 58	−24 to 55	264 ± 73	297 ± 88	−74 to 9

Values are means ± SD; CI, 95% confidence interval of differences; GM, gastrocnemius medialis; SOL, soleus; *$P < 0.05$ *comparing raw values pre- and post-test,* §$P < 0.05$ *comparing values normalized to instantaneous MTU length pre- and post-test,* ¤ $P < 0.05$ *comparing baseline, raw values between the groups. NB: When normalized to MTU length, all variables were similar between groups at baseline.*

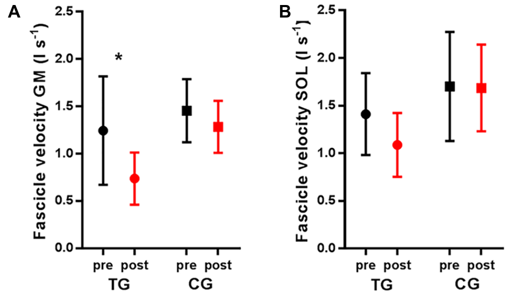

FIGURE 3 | Average fascicle velocity in the training group (TG) and the control group (CG) measured during pre-test (pre) and post-test (post). **(A)** Gastrocnemius medialis (GM) and **(B)** soleus (SOL) fascicle velocities were calculated as the first derivative of the normalized fascicle length. Values are means ± SD. *$P < 0.05$ comparing pre- and post-test.

significant differences were found between pre- post-training values of joint angle excursion and MTU lengthening during the landing period, the execution of the landing task was similar overall before and after training. Furthermore, the similarity between raw and normalized (i.e., to MTU length) strain data confirms that the observed changes after training were not entirely due to variability in the execution of the task.

Muscle and Tendon Mechanics During Landing

The unaltered tendon strain measured during isometric contractions after training is congruent with the similar tendon elongation observed during landing, despite unchanged joint moments. As mentioned above (see *Changes in muscle–tendon properties*), a type II error cannot be entirely ruled out, but an insufficient increase in stiffness to constrain tendon strain seems a more likely explanation. It can be speculated that the increase in stiffness (<100 Nmm^{-1}) found in this study was not sufficient to substantially reduce tendon longitudinal strain during landing. Alternatively, recent studies have highlighted the complexity of tendon deformation (Farris et al., 2013; Raiteri et al., 2016), and in particular the two-dimensional nature of this deformation in the proximal part of the Achilles tendon. The present intervention may have resulted in direction-specific changes in tendon mechanical properties, limiting deformation in the transverse direction. Likewise, the structural changes of the muscle due to hypertrophy may have resulted in different two-dimensional strain patterns in the Achilles tendon. Future studies looking at the links between tendon properties and function should include transverse strain measurements to verify these hypotheses.

Despite the lack of significant change in longitudinal deformation of the tendon, the active lengthening of the gastrocnemius fascicles during landing was reduced after training. A similar trend was found in soleus, but this disappeared after accounting for MTU length changes, suggesting distinct roles of the two muscles or a smaller effect of training on soleus. Here again, region-specific changes in tendon strain patterns or mechanical properties may have limited the effect of training on the free tendon and therefore the soleus contractile behavior. In support of this possibility, recently published modeling data demonstrate the reduced sensitivity of soleus muscle–tendon mechanics to changes in tendon compliance compared to gastrocnemius (Orselli et al., 2017). Consistent with the data on fascicle lengthening during landing, gastrocnemius fascicle lengthening velocity was also reduced after training. A slower active lengthening of the fascicles does not provide any advantage for force production, but may mitigate the damage caused by faster eccentric contractions (Proske and Morgan, 2001).

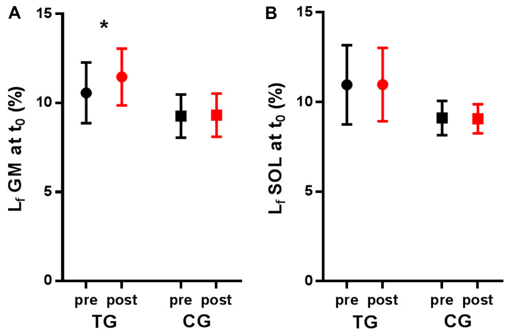

FIGURE 4 | Fascicle length at touch down in the training group (TG) and the control group (CG) measured during pre-test (pre) and post-test (post). **(A)** Gastrocnemius medialis (GM) and **(B)** soleus (SOL) fascicle lengthening were normalized to the respective MTU lengths. $^{*}P < 0.05$ comparing pre- and post-test.

In the case of the gastrocnemius muscle, the offset toward longer fascicle length at touch down is noteworthy. Initial fascicle length may have conditioned lengthening magnitude if this parameter was determined by the final standing position (i.e., at the end of the landing phase). Owing to the gastrocnemius force-length relationship, the increased fascicle length may have increased the force produced at a similar level of muscle activation, which could partly explain the reduction in fascicle lengthening after training. Another possible reason for this offset is the variability in fascicle segmentation on the first image of each ultrasound scanning sequence. A recent analysis of the reliability of fascicle tracking (Aeles et al., 2017) found good reliability for the analysis of fascicle length changes but also pointed at the more variable segmentation of the initial image, rendering the comparison of absolute lengths more challenging. However, in the present study, the same investigator (AW) analyzed all images in a random order and the fact that no differences in fascicle length at touch down were found in the control group support these findings. So, is this offset induced by training or simply linked to the reduction in ankle plantarflexion at touchdown? The latter interpretation seems unlikely here, because the same analyses performed on the values normalized to instantaneous MTU length confirmed that fascicles were longer at the beginning of the landing (**Figure 4**). Since training did not induce any change in resting fascicle length, a possible explanation for the differences seen at touch down could lie in the stiffening of elastic structures proximal to the Achilles tendon (i.e., proximal tendon, aponeurosis, connective tissue), which would constrain fascicles to a longer length at touch down. This hypothesis would also be consistent with the delayed onset of fascicle lengthening after training, because of an increased resistance of elastic tissue. It would additionally be compatible with the fact that the offset was seen for the gastrocnemius but not for the soleus muscle.

Energy Storage and Dissipation

The fact that the task and the mass of the subjects were identical before and after the intervention implies that requirements for energy storage and dissipation were unchanged. However, in the trained subjects, the increased plantarflexion strength combined with a stiffer Achilles tendon that underwent the same elongation after training, indicates that more energy was being stored during landing. Had fascicles produced more force, a similar amount of energy would have been dissipated despite a reduced lengthening. Increased fascicle force production would have been consistent with the observed muscle hypertrophy. It would also be in line with the longer operating length of the fascicles after training, since the triceps surae muscles operate on the ascending limb of the force-length relationship (Maganaris, 2003a). However, such an increase is incompatible with our calculations of tendon force during landing, which did not change after training. This point and the unaltered work done at the ankle and knee joints make the interpretation of the findings difficult in relation to the energetic consequences of the observed changes. Of course, the accuracy of the estimated tendon force is limited by a number of assumptions, amongst which antagonist co-activation level was similar between subjects before and after the intervention. Despite the limited predictive value of electromyographic amplitude to estimate antagonist torque, future studies may use other *in vivo* solutions to improve force estimation. For the time being, the unaltered Achilles tendon force and work done at the ankle and knee joints make the interpretation of the findings difficult in relation to the energetic consequences of the observed changes.

CONCLUSION

This study showed that training-induced increases in Achilles tendon stiffness and muscle strength did not affect the buffering capacity of the tendon against large fascicle strain during landing. On the contrary, decreases in gastrocnemius fascicle lengthening and lengthening velocity were observed after training, although it could not be directly linked to unchanged longitudinal strain of the tendon. Questions remain regarding the mechanisms underpinning the observed changes in fascicle behavior, and further research is warranted to assess more precisely the impact of these changes on energy dissipation. The techniques currently used to study muscle–tendon mechanics *in vivo* are limited by necessary assumptions and possible associated errors. Reducing the likelihood and effects of these errors will allow the present findings to be confirmed and built upon.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the institutional ethical committee, Ethical Committee of the Norwegian School of Sport Sciences. The protocol was approved by the Ethical Committee of the

Norwegian School of Sport Sciences. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

AW and OS performed the experiments, analyzed the data, and drafted the manuscript. AW, KA, NC, GP, JB-M, and OS were all involved in the conception and design of the research and edited, revised, and approved the 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.2018.00794/full#supplementary-material

REFERENCES

Aeles, J., Lichtwark, G. A., Lenchant, S., Vanlommel, L., Delabastita, T., and Vanwanseele, B. (2017). Information from dynamic length changes improves reliability of static ultrasound fascicle length measurements. *PeerJ* 5:e4164. doi: 10.7717/peerj.4164

Albracht, K., and Arampatzis, A. (2013). Exercise-induced changes in triceps surae tendon stiffness and muscle strength affect running economy in humans. *Eur. J. Appl. Physiol.* 113, 1605–1615. doi: 10.1007/s00421-012-2585-4

Arampatzis, A., Karamanidis, K., and Albracht, K. (2007). Adaptational responses of the human Achilles tendon by modulation of the applied cyclic strain magnitude. *J. Exp. Biol.* 210, 2743–2753. doi: 10.1242/jeb.003814

Arampatzis, A., Morey-Klapsing, G., Karamanidis, K., DeMonte, G., Stafilidis, S., and Brüggemann, G.-P. (2005). Differences between measured and resultant joint moments during isometric contractions at the ankle joint. *J. Biomech.* 38, 885–892. doi: 10.1016/j.jbiomech.2004.04.027

Arampatzis, A., Peper, A., Bierbaum, S., and Albracht, K. (2010). Plasticity of human Achilles tendon mechanical and morphological properties in response to cyclic strain. *J. Biomech.* 43, 3073–3079. doi: 10.1016/j.jbiomech.2010.08.014

Balshaw, T. G., Massey, G. J., Maden-Wilkinson, T. M., Tillin, N. A., and Folland, J. P. (2016). Training-specific functional, neural, and hypertrophic adaptations to explosive-vs. sustained-contraction strength training. *J. Appl. Physiol.* 120, 1364–1373. doi: 10.1152/japplphysiol.00091.2016

Cormie, P., McGuigan, M. R., and Newton, R. U. (2010). Influence of strength on magnitude and mechanisms of adaptation to power training. *Med. Sci. Sports Exerc.* 42, 1566–1581. doi: 10.1249/mss.0b013e3181cf818d

Cronin, N. J., Carty, C. P., Barrett, R. S., and Lichtwark, G. (2011). Automatic tracking of medial gastrocnemius fascicle length during human locomotion. *J. Appl. Physiol.* 111, 1491–1496. doi: 10.1152/japplphysiol.00530.2011

Dick, T. J. M., Arnold, A. S., and Wakeling, J. M. (2016). Quantifying Achilles tendon force *in vivo* from ultrasound images. *J. Biomech.* 49, 3200–3207. doi: 10.1016/j.jbiomech.2016.07.036

Farris, D. J., and Lichtwark, G. A. (2016). UltraTrack: software for semi-automated tracking of muscle fascicles in sequences of B-mode ultrasound images. *Comput. Methods Programs Biomed.* 128, 111–118. doi: 10.1016/j.cmpb.2016.02.016

Farris, D. J., Trewartha, G., McGuigan, M. P., and Lichtwark, G. A. (2013). Differential strain patterns of the human Achilles tendon determined *in vivo* with freehand three-dimensional ultrasound imaging. *J. Exp. Biol.* 216, 594–600. doi: 10.1242/jeb.077131

Fukashiro, S., Komi, P. V., Järvinen, M., and Miyashita, M. (1993). Comparison between the directly measured Achilles tendon force and the tendon force calculated from the ankle joint moment during vertical jumps. *Clin. Biomech.* 8, 25–30. doi: 10.1016/S0268-0033(05)80006-3

Gerus, P., Rao, G., and Berton, E. (2011). A method to characterize *in vivo* tendon force–strain relationship by combining ultrasonography, motion capture and loading rates. *J. Biomech.* 44, 2333–2336. doi: 10.1016/j.jbiomech.2011.05.021

Gillett, J. G., Barrett, R. S., and Lichtwark, G. A. (2013). Reliability and accuracy of an automated tracking algorithm to measure controlled passive and active muscle fascicle length changes from ultrasound. *Comput. Methods Biomech. Biomed. Eng.* 16, 678–687. doi: 10.1080/10255842.2011.633516

Hawkins, D., and Hull, M. L. (1990). A method for determining lower extremity muscle-tendon lengths during flexion/extension movements. *J. Biomech.* 23, 487–494. doi: 10.1016/0021-9290(90)90304-L

Hirayama, K., Iwanuma, S., Ikeda, N., Yoshikawa, A., Ema, R., and Kawakami, Y. (2017). Plyometric training favors optimizing muscle–tendon behavior during depth jumping. *Front. Physiol.* 8:16. doi: 10.3389/fphys.2017.00016

Karamanidis, K., Stafilidis, S., DeMonte, G., Morey-Klapsing, G., Brüggemann, G.-P., and Arampatzis, A. (2005). Inevitable joint angular rotation affects muscle architecture during isometric contraction. *J. Electromyogr. Kinesiol.* 15, 608–616. doi: 10.1016/j.jelekin.2005.02.001

Kubo, K., Kanehisa, H., and Fukunaga, T. (2002). Effects of resistance and stretching training programmes on the viscoelastic properties of human tendon structures *in vivo*. *J. Physiol.* 538(Pt 1), 219–226. doi: 10.1113/jphysiol.2001.012703

Kwah, L. K., Pinto, R. Z., Diong, J., and Herbert, R. D. (2013). Reliability and validity of ultrasound measurements of muscle fascicle length and pennation in humans: a systematic review. *J. Appl. Physiol.* 114, 761–769. doi: 10.1152/japplphysiol.01430.2011

Lichtwark, G. A., and Wilson, A. M. (2007). Is Achilles tendon compliance optimised for maximum muscle efficiency during locomotion? *J. Biomech.* 40, 1768–1775. doi: 10.1016/j.jbiomech.2006.07.025

Lichtwark, G. A., and Wilson, A. M. (2008). Optimal muscle fascicle length and tendon stiffness for maximising gastrocnemius efficiency during human walking and running. *J. Theor. Biol.* 252, 662–673. doi: 10.1016/j.jtbi.2008.01.018

Maganaris, C. N. (2003a). Force-length characteristics of the in vivo human gastrocnemius muscle. *Clin. Anat.* 16, 215–223. doi: 10.1002/ca.10064

Maganaris, C. N. (2003b). Tendon conditioning: artefact or property? *Proc. R. Soc. Lond. B Biol. Sci.* 270(Suppl. 1), S39–S42. doi: 10.1098/rsbl.2003.0004

Miller, B. F., Olesen, J. L., Hansen, M., Døssing, S., Crameri, R. M., Welling, R. J., et al. (2005). Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. *J. Physiol.* 567(Pt 3), 1021–1033. doi: 10.1113/jphysiol.2005.093690

Obst, S. J., Barber, L., Miller, A., and Barrett, R. S. (2017). Reliability of Achilles tendon moment arm measured in vivo using freehand three-dimensional ultrasound. *J. Appl. Biomech.* 33, 300–304. doi: 10.1123/jab.2016-0261

Orselli, M. I. V., Franz, J. R., and Thelen, D. G. (2017). The effects of Achilles tendon compliance on triceps surae mechanics and energetics in walking. *J. Biomech.* 60, 227–231. doi: 10.1016/j.jbiomech.2017.06.022

Proske, U., and Morgan, D. L. (2001). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J. Physiol.* 537, 333–345. doi: 10.1111/j.1469-7793.2001.00333.x

Raiteri, B. J., Cresswell, A. G., and Lichtwark, G. A. (2016). Three-dimensional geometrical changes of the human tibialis anterior muscle and its central aponeurosis measured with three-dimensional ultrasound during isometric contractions. *PeerJ* 4:e2260. doi: 10.7717/peerj.2260

Roberts, T. J., and Azizi, E. (2010). The series-elastic shock absorber: tendons attenuate muscle power during eccentric actions. *J. Appl. Physiol.* 109, 396–404. doi: 10.1152/japplphysiol.01272.2009

Roberts, T. J., and Azizi, E. (2011). Flexible mechanisms: the diverse roles of biological springs in vertebrate movement. *J. Exp. Biol.* 214, 353–361. doi: 10.1242/jeb.038588

Roberts, T. J., and Konow, N. (2013). How tendons buffer energy dissipation by muscle. *Exerc. Sport Sci. Rev.* 41, 186–193. doi: 10.1097/jes.0b013e3182a4e6d5

Seynnes, O. R., Bojsen-Møller, J., Albracht, K., Arndt, A., Cronin, N. J., Finni, T., et al. (2015). Ultrasound-based testing of tendon mechanical properties: a critical evaluation. *J. Appl. Physiol.* 18, 133–141. doi: 10.1152/japplphysiol.00849.2014

Seynnes, O. R., Erskine, R. M., Maganaris, C. N., Longo, S., Simoneau, E. M., Grosset, J. F., et al. (2009). Training-induced changes in structural and mechanical properties of the patellar tendon are related to muscle hypertrophy but not to strength gains. *J. Appl. Physiol.* 107, 523–530. doi: 10.1152/japplphysiol.00213.2009

Timmins, R. G., Shield, A. J., Williams, M. D., Lorenzen, C., and Opar, D. A. (2016). Architectural adaptations of muscle to training and injury: a narrative review outlining the contributions by fascicle length, pennation angle and muscle thickness. *Br. J. Sports Med.* doi: 10.1136/bjsports-2015-094881 [Epub ahead of print].

Werkhausen, A., Albracht, K., Cronin, N. J., Meier, R., Bojsen-Møller, J., and Seynnes, O. R. (2017). Modulation of muscle–tendon interaction in the human triceps surae during an energy dissipation task. *J. Exp. Biol.* 220:4141. doi: 10.1242/jeb.164111

Wiesinger, H.-P., Kösters, A., Müller, E., and Seynnes, O. R. (2015). Effects of increased loading on *in vivo* tendon properties: a systematic review. *Med. Sci. Sports Exerc.* 47, 1885–1895. doi: 10.1249/mss.0000000000000603

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Article 3

Distinct muscle-tendon interaction during running at different speeds and in different loading conditions

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Abstract

The interaction between the Achilles tendon and the triceps surae muscles seems to be modulated differently when various external constraints are altered to increase work requirements. These adjustments are often assumed to benefit efficient work production through an increased utilization of elastic energy. Thus, we tested the hypothesis that the increased need for mechanical work during running under contrasting conditions (altered speed or load) would be met by different adjustments at the muscle-tendon level, by increasing muscle activity or elastic energy utilization, respectively. Ultrasonography, electromyography, kinematics and ground reaction force measurements were used to examine Achilles tendon, gastrocnemius and soleus muscles mechanics in sixteen runners in four different running conditions, consisting of a combination of two different speeds (preferred and +20% of preferred speed) and two loading conditions (unloaded and +20% of body mass). Joint work increased similarly (+13%) with speed and load. With increasing running speed, muscle electromyographic activity increased in gastrocnemius (+10%, $P<0.01$) and soleus (+14%, $P<0.01$), but tendon length changes were unaltered. Conversely, when loading was increased, muscle activity was unaffected but tendon recoil increased (+29%, $P<0.01$). Gastrocnemius and soleus muscle fascicle length and velocity were not altered by either condition, suggesting that contractile conditions are preserved despite the constraints imposed in this experimental design. However, these results show distinct adjustments of muscle-tendon mechanics to increased speed and loading conditions during running. They support the theory of preferential utilization of elastic energy over muscle work when running conditions are favorable to it.

New and Noteworthy

We demonstrate that muscle-tendon mechanics are adjusted differently under constraints imposed by speed or load, despite comparable increases in work. Energy availability and time constraints are factors likely modulating muscle-tendon interaction, favoring elastic energy utilization over higher muscle activity. Similar gastrocnemius and soleus fascicle behavior across conditions further suggests a prioritization within the muscle-tendon unit to conserve contractile conditions when the demands of running are changed.

Running head

Muscle-tendon mechanics under differing running constraints

Keywords

Achilles tendon, running, load carriage, locomotion, muscle architecture

Introduction

Tendons play an essential role during locomotor tasks such as running (4, 21). During stance, tendons lengthen under load, uncoupling fascicle behavior from that of the muscle-tendon unit (MTU) and enabling elastic energy storage and release to accelerate the body forward during push-off. This mechanism offers several advantages, allowing muscle fascicles to operate under favorable conditions, recycling mechanical energy and amplifying the MTU power output during recoil (27). Importantly, the interaction between muscle and tendon can be modulated according to external constraints, as previously demonstrated with a change in running slope (20) or in the transition from walking to running (9). Running conditions that alter the availability of energy and time may require tuning of the rate of force development and ground reaction force impulse via changes in muscle-tendon interaction. It follows that when joint work requirements increase, muscle-tendon unit force can be increased efficiently by favoring tendon strain and elastic energy utilization over metabolic energy. This notion seems to be supported by studies reporting an unchanged shortening velocity of the gastrocnemius (9) and soleus (16) muscles during running at incremental speeds (up to 5 ms^{-1}), despite shorter ground contact durations and higher MTU shortening velocities. Additionally, although triceps surae muscles may operate at shorter lengths and at higher activation levels with increasing running speed (14, 16), a greater stretch of the elastic elements seems to preserve their relative elastic energy contribution to MTU work. However, direct measurements characterising the behaviour of elastic tissue – in particular the Achilles tendon – under increased speed conditions have to date never been reported.

Another paradigm to study MTU strategies to increase work efficiently involves increasing inertial load. This experimental design is complementary to the approach of increasing speed, because it also requires an increase in work (22) but under different time and energy constraints. Contrary to conditions imposed by increasing speed, loaded running increases the availability of potential energy, prolongs ground contact time and increases peak forces (28), implying a greater ground impulse. During jumping, Wade and colleagues showed in an elegant study how the work contribution of the ankle joint remains constant when total work is increased via additional loading, whereas it is decreased when higher work is imposed by increasing jump height (30). The authors further suggested that the use of additional elastic strain energy stored in the Achilles tendon is prioritized over muscle work during submaximal jumps with loading (30). Here also, direct measurements of muscle and tendon behavior may allow this theory to be confirmed and extended to running.

The aim of the present study was therefore to investigate the strategies used to increase work production during running, by looking at the behavior of the human triceps surae muscles and the Achilles tendon in response to increased loading or speed. Based on findings from other locomotor tasks with varying energy availability (9, 30) and contrasting time constraints, different strategies to increase positive work (i.e. force, assuming similar kinematics) at the ankle were expected when increasing speed versus increasing load. At increased running speed, the ground contact time available for force production is reduced. We expected greater muscle activity to reflect a higher rate of force development and higher peak forces, possibly causing a higher Achilles tendon strain and a reduction in fascicle operating length. Contrarily, when applying additional loading at preferred running speed, we hypothesized that greater tendon loading would be achieved over a longer stance duration. The increased utilization of elastic energy would require a smaller increase in muscle activity under this condition, representing a more efficient strategy to increase work (26).

Materials and Methods

Subjects and experimental protocol

Data were collected from sixteen male distance runners (age = 27±4 years, height = 1.79±0.05 m, mass = 68±6 kg) who ran at least 40 km per week. A warm up period of five minutes barefoot running on an instrumented treadmill (M-Gait, Motekforce Link, Amsterdam, The Netherlands) was used to determine the individual preferred speed of each subject. Thereafter the subjects were asked to run at their individual preferred speed and at increased speed (+ 20% of the preferred speed), with and without additional loads (+ 20% of body weight). Loading was achieved by means of one or two adjustable weighted vests containing up to 10 kg each. Data were recorded for at least 10 complete steps during each of the four conditions. Ultrasound, kinematic and kinetic data were synchronously collected from the right leg, while muscle activity was recorded from the left leg at the same time. All measurements were synchronized during acquisition with a trigger signal sent from the ultrasound apparatus. All tests were performed twice, to obtain ultrasound scans from the muscle fascicles and from the myotendinous junction. The protocol was approved by the ethical committee of the Norwegian School of Sport Sciences and all subjects gave written informed consent to participate in the study.

Joint mechanics

Eleven infrared cameras (Qualisys, Gothenburg, Sweden; 300 Hz) captured the three-dimensional position of 20 reflective markers, mounted on the right leg of the subjects. Reflective markers placed over relevant anatomical landmarks (right and left anterior and posterior iliac spine, medial and lateral condyles, medial and lateral malleoli, calcaneus, first, second and fifth metatarsal) were used to define the joint centers of the pelvis (3), the right knee and the right ankle. The same calibration markers were used to define local coordinate systems for the body segments (pelvis, thigh, shank and foot) during a static capture. While calibration markers of the pelvis and foot were also used as tracking markers, the movement of the shank and thigh were tracked with 4-marker clusters positioned mid-way along these segments. A force plate instrumented to the treadmill measured ground reaction forces during the running trials. Initial ground contact and toe-off were defined with a threshold of 25N. Inverse kinematic and dynamic calculations were performed for the right leg using Visual 3D (C-motion, Germantown, MD). Negative and positive ankle and knee joint work were calculated by integrating the negative and positive joint power using trapezoidal integration. Individual net-work was calculated as the difference between negative and positive work for both joints. Joint angle data were used to estimate MTU lengths of gastrocnemius and soleus (11).

Muscle-tendon mechanics

Gastrocnemius and soleus muscle fascicles and the gastrocnemius muscle-tendon junction were imaged using an ultrasound linear array transducer (LV7.5/60/96Z LS128, Telemed, Vilnius, Lithuania). The transducer was secured to the leg in a custom-made holder with self-adhesive tape to avoid probe movement. Tape was also used to rigidly attach three kinematic markers to the transducer to track its position. B-mode images with a field of view of 60 mm were collected at 80 frames s^{-1}. To measure fascicle length and pennation angle, the transducer was placed over the gastrocnemius muscle belly and aligned with the azimuthal direction of fascicles. A semi-automated tracking algorithm was used offline to analyze fascicle lengths and pennation angles (6, 8). The image quality of the soleus scans of three subjects was insufficient for analysis. Consequently, data for this muscle are based on thirteen subjects instead of sixteen.

The position of the muscle-tendon junction was tracked from the displacement of the closest visible fascicle insertion in the two-dimensional ultrasound images (Tracker 4.95; www.physlets.org/tracker/). Applying previously obtained calibration of the image coordinate system and the transducer coordinate system, the position of the muscle-tendon junction was reconstructed into the three-dimensional coordinate system of the laboratory (19). Thus, the distance between the gastrocnemius muscle-tendon junction and a reflective marker over the osteotendinous junction on the calcaneus was defined as Achilles tendon length. The position of the osteotendinous junction of the Achilles tendon was previously identified using ultrasound. The shortest perpendicular distance between the force vector of the Achilles tendon and the ankle joint center was defined as the tendon moment arm at each time point (24). Thus, Achilles tendon force was estimated by dividing the ankle moment by the moment arm of the tendon.

Muscle activity

Muscle activity of gastrocnemius, soleus and tibialis anterior was measured with a wireless electromyography (EMG) system (TeleMyo DTS, Noraxon U.S.A. Inc., Scottsdale, AZ, USA) and recorded in Qualisys. Surface electrodes were placed on the muscle belly following SENIAM guidelines (12), after the recording sites were shaved and cleaned. EMG signals were collected at 1500 Hz and processed using a bandpass filter (20 – 450 Hz), rectified and low-pass filtered (10 Hz). For each subject and muscle, EMG data were normalized to the highest values recorded during the control trials (i.e. without added mass and at preferred speed).

Data processing and statistics

Kinematic, kinetic, ultrasound and EMG data were acquired during ten strides and at least eight strides were included in the analysis for each subject and each condition (9.5 ± 0.6 strides). All data were visually inspected to identify and exclude strides when their pattern or excursions departed from most other strides. A bidirectional second order Butterworth filter with a 15 Hz cut-off was applied to all raw data (except EMG, see above). Velocities of relevant variables were calculated as their time differential. Data were synchronized and resampled across full stride cycles starting with the right heel strike, to calculate means for individual subjects and conditions.

Descriptive statistics were calculated for all variables and differences between conditions were tested with two-way repeated-measures ANOVAS (factors: speed and load) and Sidak multiple comparison tests as appropriate. For all statistical tests, alpha was set to 0.05.

Results

The average preferred and increased running speeds were 3.1 (± 0.3) m s^{-1} (± 0.3) and 3.7 (± 0.3) m s^{-1}, respectively. Step cycle and stance phase durations were affected by load and speed conditions. When speed increased, cycle duration decreased in both the unloaded and loaded conditions (-4 to -6%). Adding load also resulted in a reduction of cycle duration at increased speed (-3%) but not at preferred speed (**Table 1**). Stance duration decreased when running speed was increased, with or without additional load. On the contrary, stance duration was longer when the subjects ran with added mass at either speed (**Figure 1A**). Stance duration did not differ between running at increased speed with load and running at preferred speed unloaded. For this reason, average stance durations for these two conditions are indicated with the same shade of grey in the figures.

Table 1. *Durations of step cycle and stance phase, positive work performed at the ankle and the knee, peak shortening velocities of the Achilles tendon, muscle-tendon units and fascicles, and AT forces, when running unloaded or loaded, at preferred speed or increased speed.*

			Unloaded		**Loaded**	
			Preferred speed	**Increased speed**	**Preferred speed**	**Increased speed**
Duration [s]		**Cycle**	0.68 ± 0.04	0.66 ± 0.05§	0.68 ± 0.05	0.64 ± 0.05*§
		Stance	0.23 ± 0.02	0.21 ± 0.02§	0.26 ± 0.02*	0.23 ± 0.02*§
Work [J]	**positive**	**Ankle**	55 ± 13	62 ± 15§	62 ± 18*	69 ± 20*§
		Knee	14 ± 8	16 ± 10	17 ± 9*	19 ± 10*
	negative	**Ankle**	32 ± 14	36 ± 16§	37 ± 18*	43 ± 20*§
		Knee	27 ± 11	29 ± 12	31 ± 11*	31 ± 11
	net	**Ankle**	23 ± 6	26 ± 7	25 ± 10	26 ± 11
		Knee	-13 ± 7	-13 ± 5	-14 ± 9	-12 ± 6
Peak shortening velocity [mms^{-1}]		**AT**	185 ± 42	199 ± 42	213 ± 60*	283 ± 77*§
		GM MTU	689 ± 118	779 ± 96§	607 ± 139*	765 ± 111§
		SOL MTU	656 ± 120	760 ± 103§	570 ± 128*	727 ± 99§
		GM fasc.	130 ± 60	133 ± 46	141 ± 73	147 ± 72
		SOL fasc.	115 ± 49	125 ± 51	106 ± 34	123 ± 43
Force [N]		**AT**	4336 ± 931	4644 ± 1037§	4501 ± 1029*	4896 ± 1059*§
Impulse [Ns]			469 ± 59	452 ± 61§	560 ± 76*	529 ± 75*§

Data for work, shortening velocities of the Achilles tendon (AT), gastrocnemius (GM) and soleus (SOL) muscle-tendon units (MTU) and fascicles, and force were obtained from the stance phase. Values are mean ± SD. *Significantly different from the unloaded condition at the same speed. §Significantly different from the preferred speed condition with the same load.

Joint and muscle-tendon mechanics

More positive and negative work was done by the ankle joint at higher speed in both loading conditions and with increased loading in both speed conditions. The positive work performed at the knee joint also increased when subjects were loaded (irrespective of speed), whereas speed did not have a statistically significant effect on knee work. Ankle and knee joint net-work did not change at any condition (**Table 1**).

Peak ankle and knee joint angles were consistent across speed and loading conditions and, accordingly, no differences were found for gastrocnemius and soleus MTU peak lengths. Likewise, the shortening magnitude of both MTUs before toe off was similar across all speed and mass conditions. However, maximum MTU shortening velocities increased with speed, regardless of loading conditions (**Table 1, Figure 1F**). At preferred speed, load carrying significantly reduced MTU velocities, whereas no difference was present at increased speed.

Mean length changes of the MTUs, fascicles and the Achilles tendon during a step are presented for all modalities of speed and mass in **Figure 2**. Neither speed nor mass had a substantial influence on changes in gastrocnemius or soleus fascicle length. Mean fascicle length during the stance phase, fascicle shortening and peak fascicle shortening velocity did not vary across speed and loading conditions for either muscle. While changes in pennation angle during stance were similar under all conditions in both muscles, mean gastrocnemius pennation was higher during the first half of stance (during negative work production at the ankle) when subjects ran with load at both speeds. None of the experimental conditions affected mean soleus pennation angle. Achilles tendon lengthening and peak length were unaffected by increases in speed or load. A trend towards higher tendon stretch velocities was observed with loading but did not quite reach significance ($P = 0.06$). However, a significant increase in tendon shortening amplitude (i.e. recoil) was found when running with load (**Figure 1B**), independently of speed, although this variable was not affected by an increase in speed. Additionally, we found an interaction of loading and speed for the peak recoil velocity of the Achilles tendon. Tendon recoil velocity was higher when subjects ran with load at either speed. Unlike recoil amplitude, increasing speed did result in an increase in recoil velocity, but only under the loaded modality (**Table 1**). Peak Achilles tendon force increased with speed in both loading conditions, and with loading at both speeds (**Table 1**).

Muscle activity

Running at higher speed increased mean muscle activity of the gastrocnemius and soleus during stance, in both loading modalities. There was no main effect of loading on muscle activity but an interaction and post-hoc tests indicated that in both muscles, mean EMG during the stance phase was depressed when running with additional loading at preferred speed whereas it was similar to unloaded condition at increased speed (**Figure 1D,E**). Activity of the antagonist tibialis anterior peaked during the swing phase in all conditions. However, the mean activity of this muscle during the stance phase increased with speed in both loading conditions, whereas load carrying did not affect tibialis anterior activity. The activity of all three muscles during the whole step cycle is presented in **Figure 3**.

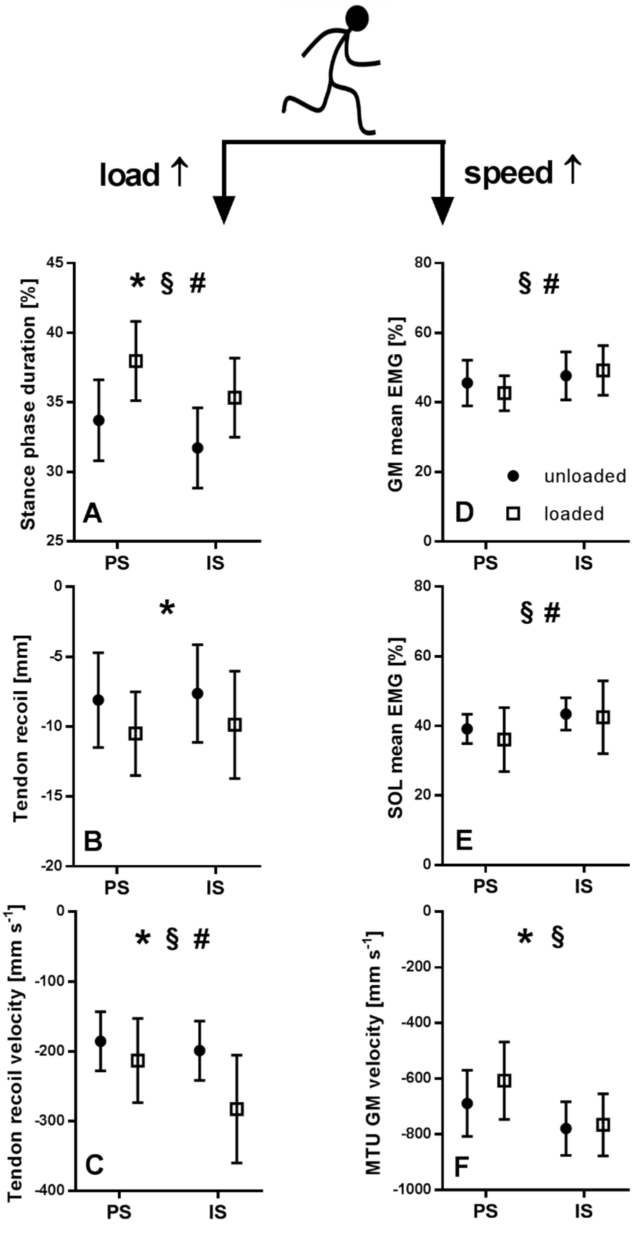

Figure 1. *Group mean values (± s.d.) during the stance phase for key variables that vary with loading or speed (preferred (PS) compared to increased speed (IS)): stance phase duration (A), Achilles tendon (AT) shortening (B), AT velocity (C), gastrocnemius (GM) EMG (D), soleus (SOL) EMG (E) and muscle-tendon unit (MTU) velocity (F). *$P<0.05$ for main effects of mass, § $P<0.05$ for main effect of speed, and # $P<0.05$ for interaction effect.*

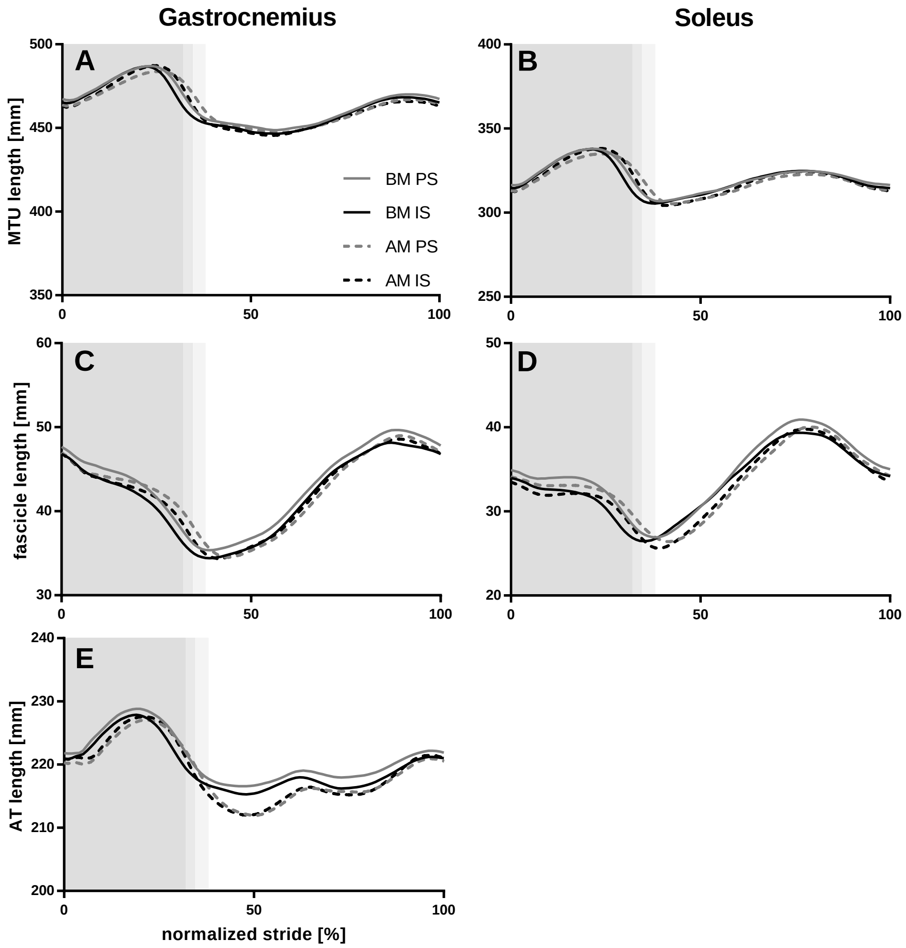

Figure 2. *Instantaneous lengths of the gastrocnemius and soleus muscle-tendon unit (MTU) (A-B), fascicles (C-D) and the Achilles tendon (AT) (E) during a whole step cycle for running at two different speeds (preferred speed - PS and increased speed - IS) and two mass conditions (body mass - BM and added mass - AM). Data are time normalized to 101 points and displayed as group means. The shaded area represents the stance phase of the conditions BM IS as dark grey, BM PS and AM IS as medium grey, and AM PS as light grey.*

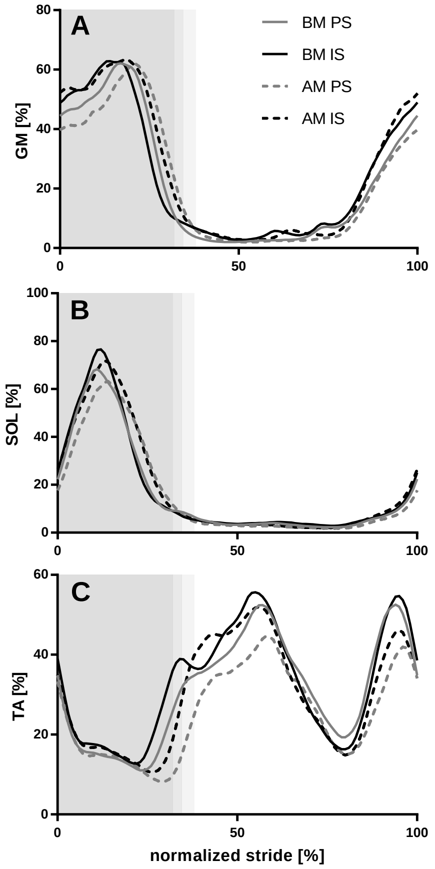

Figure 3. *Electromyographic activity of gastrocnemius medialis (GM) (A), soleus (SOL) (B) and tibialis anterior (TA) (C) during a whole step cycle for running at two different speeds (preferred speed - PS and increased speed - IS) and mass (body mass - BM and added mass - AM) conditions. Time series are normalized to 101 points and EMG values are normalized to the maximum activity during unloaded running at preferred speed. Data are displayed as group means. The shaded area represents the stance phase of the conditions BM IS as dark grey, BM PS and AM IS as medium grey, and AM PS as light grey.*

Discussion

The present study examined the effect of increased positive mechanical work of the ankle joint during running on human triceps surae muscle-tendon behavior, when either speed or loading was altered.

Based on different time constraints and availability of potential energy imposed by the two conditions, it was proposed that additional positive work at the ankle joint would be produced via distinct adjustments of muscle-tendon interaction. By virtue of conditions favoring elastic energy utilization over muscle activity, we expected a more efficient strategy with loading than with increased speed.

Consistent with predictions from the literature, higher running speed reduced the stance duration while stance was prolonged with loading. In addition, both conditions required more mechanical work at the ankle joint (as previously shown in 22). Kinematic data showed little variation, which resulted in similar MTU stretch and shortening amplitudes across all conditions. However, the analysis of muscle and tendon mechanics also showed that the greater force and work requirements were met by prioritizing different strategies in the speed and loading conditions.

The higher muscle activity of gastrocnemius and soleus found with increasing speed can be attributed to faster and higher force production when stance duration was reduced. Remarkably, the behavior of the muscle fascicles and Achilles tendon was not statistically affected by speed. In contrast, muscle activity did not change significantly with additional loading, and the increase in work and impulse was associated with a greater recoil of the Achilles tendon. Fascicle behavior was also similar under the loaded condition and a greater ground reaction impulse was produced through the prolonged stance duration and a concomitant, small increase in positive work at the knee joint. Overall (i.e. except for the mean EMG activity), the effects brought about by increasing speed were consistent in the two loading conditions and the effects of loading were consistent in the two speed conditions.

Speed and load effect on muscle fascicle behavior and activity

In the present study, gastrocnemius and soleus fascicle shortening velocities and operating lengths were similar between loading conditions. The initial stretch of fascicles measured in guinea fowl running with a similarly heavy load (23) did not occur in humans in this study. Although the loading was similar relative to body mass (22% vs 20% of body mass), the relative demands on the gastrocnemius muscle may have been higher for the guinea fowl compared to humans. If this were the case, it can be speculated that a similar fascicle stretch as in the guinea fowls could have been observed in humans at loads higher than in this study. In line with previous work examining gastrocnemius (9) and soleus (16) fascicles at different running speeds, we did not see any significant influence of speed on fascicle behavior. Despite the time constraint imposed by a higher speed (i.e. shorter ground contact duration), fascicles maintained the slow shortening velocities shown at preferred speed. Since the force that a muscle can produce at a given activation level decreases with increasing shortening velocity, an unchanged contraction velocity is beneficial for active force production (13). However, *in silico* fascicle shortening velocities are higher at higher running speeds (3.5 - 8 m/s) than those used in this study (3.1 and 3.7 m/s) (7), suggesting that fascicles may only maintain their contraction velocity within certain speed ranges. The unchanged operating length of fascicles in this study contrasts with the shift towards shorter operating lengths observed by others in the gastrocnemius (14) and soleus (16) muscles at higher running speeds. Hence, the advantage conferred to the triceps surae muscles by operating towards the top of the ascending limb of the force-length relationship may have been maintained at the speeds used in this protocol.

While fascicle velocity and length were unchanged when running at increased speed, muscle activity of gastrocnemius and soleus increased, regardless of the loading condition. The observation of increased muscle activity at higher running speed is consistent with our hypothesis and previous reports (15, 16). Conversely, gastrocnemius and soleus EMG activity was reduced when additional load was added to the runners at preferred speed. The interaction effect observed with this variable further indicates that running speed can even override the effect of loading by increasing muscle activity when both conditions are applied.

Similar adjustments in muscle fascicle behavior and muscle activity were observed within each condition for both the gastrocnemius and soleus, despite the different role and fascicle behavior of these two muscles during running (18). Fascicle behavior was preserved when running with loading or at increased speed, suggesting a prioritization of contractile conditions while other factors were adjusted to meet the increased demands. An increase in muscle activity seems to only fulfil this role when running speed is increased.

Speed and load effect on tendon behavior

Unlike the present observations, a simulation study predicted an increased elastic contribution to the positive work of the soleus and gastrocnemius MTUs when running speed increased from 2.1 to 9 ms^{-1} (17). However, the same study also suggested that the contribution of elastic strain energy of the gastrocnemius MTU would remain unchanged at intermediate speeds (17). Our results support the latter finding with direct *in vivo* measurements of Achilles tendon length, by showing that neither tendon strain nor recoil changed at speeds lower than 4 ms^{-1}. This unchanged tendon behavior suggests a rather constant contribution of elastic energy within the studied range of speed conditions.

Unlike speed changes, loading affected the Achilles tendon behavior through an increased recoil amplitude and velocity irrespective of the speed conditions. The apparent disagreement between the unaltered tendon stretch during the first part of the stance-phase and the changes in recoil during the push-off is noteworthy and may have been caused by several factors. Farris, Trewartha, McGuigan and Lichtwark (10) established that a considerable amount of energy is stored transversally in the gastrocnemius aponeurosis during isometric contractions, which concurrently reduced longitudinal strain in the Achilles tendon. In addition, aponeurosis stiffness seemingly increases proportionally with contraction force because of radially expanding muscle fascicles (1, 2), and possibly also increases with MTU length (25). In the present case, a higher aponeurosis stiffness may have limited longitudinal tendon strain, in particular at the high Achilles tendon forces occurring at long MTU length measured at mid-stance when running with load. The hypothesis of additional energy being stored through transverse strain is also compatible with the unchanged joint kinematics and muscle operating conditions observed under loading. It may in turn be seen as an advantageous way to increase work while allowing the subjects to run with the same joint coordination as in unloaded conditions and at preferred speed.

Notwithstanding the advantages of direct measurements of Achilles tendon length compared to other indirect approaches (31), methodological issues may have affected the precision of our tendon deformation measures. For instance, the fact that the Achilles tendon was modelled as a straight line may have caused an underestimation of tendon length changes (29). Although such a bias cannot fully be disregarded, its impact is deemed limited in the repeated-measure design of the present study. Finally, the mismatched changes in tendon stretch and recoil with loading may also have been caused by the longer duration of the ground contact in this condition. By allowing a larger proportion of the tendon recoil to take place before the onset of the swing phase, the greater contact time would result in an increased impulse to the ground. This hypothesis would additionally be consistent with the faster velocities of tendon recoil.

Regardless of the factors explaining the lack of change in tendon stretch, the greater magnitude of tendon recoil in the loaded conditions suggests an increase of elastic energy utilization via a larger energy return. The interaction effect seen for tendon recoil velocity may in this regard indicate that the utilization of elastic energy with loading is bound to the availability of potential energy, the duration of the stance phase and the work requirements. A higher contribution of elastic energy with loading may explain why the metabolic cost of running measured in previous experiments did not increase proportionally to the imposed loading (e.g. 5).

Conclusion

The present study showed distinct strategies of triceps surae muscle-tendon interaction to perform additional work at the ankle joint during running when speed or load increased. When this interaction was constrained at a higher running speed, by a greater mechanical demand and a shorter contact time, increased positive work was achieved with increased muscle activity. In contrast, when more energy was available to be stored and the ground contact was prolonged (i.e. with load), the utilization of elastic strain energy was prioritized. Adaptations with speed and loading were congruous across modalities, suggesting that these mechanisms come into play independently within the tested speeds. The consistent behavior of the fascicles across all conditions suggests that contractile conditions tend to be preserved within the tested ranges of speed and load, although tendon forces, ground impulse and MTU velocities changed.

References

1. **Arellano CJ, Gidmark NJ, Konow N, Azizi E, and Roberts TJ**. Determinants of aponeurosis shape change during muscle contraction. *J Biomech* 49: 1812-1817, 2016.
2. **Azizi E, and Roberts TJ**. Biaxial strain and variable stiffness in aponeuroses. *J Physiol* 587: 4309-4318, 2009.
3. **Bell AL, Brand RA, and Pedersen DR**. Prediction of hip joint centre location from external landmarks. *Hum Mov Sci* 8: 3-16, 1989.
4. **Cavagna GA, Saibene FP, and Margaria R**. Mechanical work in running. *J Appl Physiol* 19: 249-256, 1964.
5. **Cooke CB, McDonagh MJ, Nevill AM, and Davies CT**. Effects of load on oxygen intake in trained boys and men during treadmill running. *J Appl Physiol* 71: 1237-1244, 1991.
6. **Cronin NJ, Carty CP, Barrett RS, and Lichtwark G**. Automatic tracking of medial gastrocnemius fascicle length during human locomotion. *J Appl Physiol* 111: 1491-1496, 2011.
7. **Dorn TW, Schache AG, and Pandy MG**. Muscular strategy shift in human running: dependence of running speed on hip and ankle muscle performance. *J Exp Biol* 215: 1944-1956, 2012.
8. **Farris DJ, and Lichtwark GA**. UltraTrack: Software for semi-automated tracking of muscle fascicles in sequences of B-mode ultrasound images. *Comput Methods Programs Biomed* 128: 111-118, 2016.
9. **Farris DJ, and Sawicki GS**. Human medial gastrocnemius force–velocity behavior shifts with locomotion speed and gait. *PNAS* 109: 977-982, 2012.
10. **Farris DJ, Trewartha G, McGuigan MP, and Lichtwark GA**. Differential strain patterns of the human Achilles tendon determined in vivo with freehand three-dimensional ultrasound imaging. *J Exp Biol* 216: 594-600, 2013.
11. **Hawkins D, and Hull ML**. A method for determining lower extremity muscle-tendon lengths during flexion/extension movements. *J Biomech* 23: 487-494, 1990.
12. **Hermens HJ, Freriks B, Disselhorst-Klug C, and Rau G**. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol* 10: 361-374, 2000.
13. **Hill A**. The heat of shortening and the dynamic constants of muscle. *Proc R Soc Lond B Biol Sci* 126: 136-195, 1938.
14. **Ishikawa M, and Komi PV**. The role of the stretch reflex in the gastrocnemius muscle during human locomotion at various speeds. *J Appl Physiol* 103: 1030-1036, 2007.
15. **Kyröläinen H, Avela J, and Komi PV**. Changes in muscle activity with increasing running speed. *J Sports Sci* 23: 1101-1109, 2005.

16. **Lai A, Lichtwark GA, Schache AG, Lin Y-C, Brown NAT, and Pandy MG**. In vivo behavior of the human soleus muscle with increasing walking and running speeds. *J Appl Physiol* 118: 1266-1275, 2015.

17. **Lai A, Schache AG, Lin Y-C, and Pandy MG**. Tendon elastic strain energy in the human ankle plantar-flexors and its role with increased running speed. *J Exp Biol* 217: 3159-3168, 2014.

18. **Lai AKM, Lichtwark GA, Schache AG, and Pandy MG**. Differences in in vivo muscle fascicle and tendinous tissue behavior between the ankle plantarflexors during running. *Scand J Med Sci Sports* 0: 2018.

19. **Lichtwark GA, and Wilson AM**. In vivo mechanical properties of the human Achilles tendon during one-legged hopping. *J Exp Biol* 208: 4715-4725, 2005.

20. **Lichtwark GA, and Wilson AM**. Interactions between the human gastrocnemius muscle and the Achilles tendon during incline, level and decline locomotion. *J Exp Biol* 209: 4379-4388, 2006.

21. **Lichtwark GA, and Wilson AM**. Is Achilles tendon compliance optimised for maximum muscle efficiency during locomotion? *J Biomech* 40: 1768-1775, 2007.

22. **Liew BXW, Morris S, and Netto K**. The effects of load carriage on joint work at different running velocities. *J Biomech* 49: 3275-3280, 2016.

23. **McGowan CP, Duarte HA, Main JB, and Biewener AA**. Effects of load carrying on metabolic cost and hindlimb muscle dynamics in guinea fowl (Numida meleagris). *J Appl Physiol* 101: 1060-1069, 2006.

24. **Obst SJ, Barber L, Miller A, and Barrett RS**. Reliability of Achilles Tendon Moment Arm Measured In Vivo Using Freehand Three-Dimensional Ultrasound. *J Appl Biomech* 33: 300-304, 2017.

25. **Raiteri BJ, Cresswell AG, and Lichtwark GA**. Muscle-tendon length and force affect human tibialis anterior central aponeurosis stiffness in vivo. *PNAS* 2018.

26. **Roberts TJ, Kram R, Weyand PG, and Taylor CR**. Energetics of bipedal running. I. Metabolic cost of generating force. *J Exp Biol* 201: 2745, 1998.

27. **Roberts TJ, Marsh RL, Weyand PG, and Taylor CR**. Muscular Force in Running Turkeys: The Economy of Minimizing Work. *Science* 275: 1113-1115, 1997.

28. **Silder A, Besier T, and Delp SL**. Running with a load increases leg stiffness. *J Biomech* 48: 1003-1008, 2015.

29. **Stosic J, and Finni T**. Gastrocnemius tendon length and strain are different when assessed using straight or curved tendon model. *Eur J Appl Physiol* 111: 3151-3154, 2011.

30. **Wade L, Lichtwark G, and Farris DJ**. Movement Strategies for Countermovement Jumping are Potentially Influenced by Elastic Energy Stored and Released from Tendons. *Sci Rep* 8: 2300, 2018.

31. **Zelik KE, and Franz JR**. It’s positive to be negative: Achilles tendon work loops during human locomotion. *PLoS One* 12: e0179976, 2017.

Article 4

Training-induced increase in Achilles tendon stiffness affects tendon strain and muscle contraction behavior during running

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Abstract

Background. During the stance phase of running, the elasticity of the Achilles tendon enables the utilisation of elastic energy and allows beneficial contractile conditions for the triceps surae muscles. However, the effect of changes in tendon mechanical properties induced by chronic loading is still poorly understood. We tested the hypothesis that a training-induced increase in Achilles tendon stiffness would result in reduced tendon strain during the stance phase of running, which would alter fascicle behaviour of the triceps surae muscles, particularly in the mono-articular soleus.

Methods. Eleven subjects were assigned to a training group performing isometric single-leg plantarflexion contractions three times per week for ten weeks, and ten subjects formed a control group. Before and after the training period, Achilles tendon stiffness was measured, and muscle-tendon mechanics were assessed during running at preferred speed using ultrasonography, kinematics and kinetics.

Results. Achilles tendon stiffness increased by 18% ($P < 0.01$) in the training group, but the associated reduction in strain seen during isometric contractions was not statistically significant. Tendon elongation during the stance phase of running was similar after training but tendon recoil was reduced by 30% ($P < 0.01$), while estimated tendon force remained unchanged. Although neither gastrocnemius nor soleus fascicle shortening during stance was affected by training, increased angular excursion of fascicles and architectural gear ratio in the gastrocnemius indicated altered muscle mechanics for this muscle.

Discussion. These results show that a training-induced increase in Achilles tendon stiffness altered muscle and tendon behaviour during running. Despite training-induced changes in tendon mechanical properties and recoil behaviour, the data suggest that fascicle shortening patterns were preserved for the running configuration that we examined. The asymmetrical changes in tendon strain patterns and the increased gastrocnemius gear ratio support the notion that simple in series models do not fully explain the mechanical output of the muscle-tendon unit during a complex task like running.

Introduction

The integrated function of tendon and muscle plays an important role for force production during locomotion. The viscoelastic nature of the Achilles tendon enables energy conservation by uncoupling muscle fascicle behaviour from that of the muscle-tendon unit (MTU), which allows beneficial contractile conditions for the triceps surae muscles and favours economical force production (Roberts et al. 1997). It follows that changes in Achilles tendon stiffness potentially have important consequences for both tendon elastic energy storage and muscle contractile behaviour during running. While research has focused on how muscle adaptations influence force production, a better understanding of the role of tendon stiffness during running is required, especially when considering the significant changes in tendon mechanical properties that occur with loading (Wiesinger et al. 2015), ageing (McCrum et al. 2018) or injury (Obst et al. 2018). Cross-sectional comparison studies have shown differences in Achilles tendon stiffness between athletes with contrasting running specialisation (Arampatzis et al. 2007) or performance (Kubo et al. 2015) but longitudinal data supporting a causative link are scarce.

Simulation studies suggest that varying tendon stiffness affects gastrocnemius muscle mechanical work and metabolic energy consumption (Lichtwark & Wilson 2007; Lichtwark & Wilson 2008). On the basis of a two-dimensional model of the Achilles tendon in series with the gastrocnemius muscle, a change in tendon stiffness would affect the functional range of fascicles (i.e. fascicle operating length and shortening velocity), which would in turn alter the conditions of muscle work production. Contrary to this assumption, Albracht & Arampatzis (2013) did not find any alteration in gastrocnemius fascicle behaviour after a training-induced increase in Achilles tendon stiffness. However, in this study the calculated strain of the series elastic elements (SEE) during running was unaltered after training, despite the reduction in tendon strain observed during isometric contractions. This discrepancy may have been caused by the complexity and number of elastic elements reflected by SEE length calculations, which may not inform about tendon strain accurately enough. Specific measurements of Achilles tendon length may be necessary to ascertain that this type of intervention does not affect strain patterns during running. The lack of adjustment in gastrocnemius fascicle behaviour found by these authors is nonetheless noteworthy, and may be attributable to the bi-articular nature of this muscle. While lengthening of the gastrocnemius MTU is partly offset by knee flexion during the first part of the stance phase, the relatively greater lengthening of the soleus MTU and SEE (Lai et al. 2018) caused by ankle dorsiflexion suggests that length changes in this muscle may be more affected by tendon stiffening. We investigated how a training-induced increase in Achilles tendon stiffness affects the strain patterns of the Achilles tendon and of the fascicles of gastrocnemius and soleus muscles during running. We hypothesized that, while joint kinematics and kinetics would remain similar, a training-induced increase in Achilles tendon stiffness would reduce Achilles tendon strain and recoil during running. Additionally, we expected that the change in tendon properties would affect muscle fascicle length changes, primarily in the soleus, owing to the mono-articularity of this muscle and the associated differences in architecture and contractile behaviour in comparison to the gastrocnemii.

Methods

Subjects and experimental design

The experiment was performed on 21 recreationally active adults, as a part of a larger study partly published elsewhere (Werkhausen et al. 2018). Volunteers were excluded from the study if they reported any injury or a history of systematic lower leg strength training. Eleven subjects were assigned to the training group (height 174 cm, body mass 70 kg, age 26 years, five men and six women) and ten subjects served as controls (height 178 cm, body mass 73 kg, age 30 years, six men and four women). All subjects were fully informed about the experiment and provided written informed consent to participate in this study. The protocol was approved by the Ethical Committee of the Norwegian School of Sport Sciences (14-220817).

The training group took part in a resistance training program focused on plantarflexor muscles for ten weeks, while the control group did not change their daily activities during this period. All subjects were tested similarly before and after the training period. All tests were performed barefoot and on the right leg only. The warm up for the testing sessions consisted of 5 minutes barefoot running on a treadmill at individual preferred speed. The testing procedure included two experiments using ultrasound, kinematic and kinetic measurements synchronized by a trigger signal sent by the ultrasound device. Firstly, Achilles tendon stiffness, plantarflexion strength and resting gastrocnemius muscle architecture were measured. Secondly, *in vivo* muscle-tendon behaviour during running at preferred speed (2.8 ± 0.4 ms^{-1} and 2.3 ± 0.2 ms^{-1} for the training group and the controls, respectively, as determined during the first test) were examined. The running test was performed twice, to acquire images of the muscle-tendon junction and of the muscle fascicles.

Training program

The training group performed 4 x 10, unilateral, isometric plantarflexion contractions three times per week for ten weeks. The exercise was always preceded by a five-minute easy warm-up on a cycle ergometer. As the aim was to increase Achilles tendon stiffness while minimising adaptations of muscle architecture and strength, contractions were performed explosively at a fixed joint angle and were maintained for one second followed by five seconds of rest. Plantarflexion training was performed in a standing position in a custom-built rig (Werkhausen et al. 2018). The training device was adjusted for every subject so that the ankle was in anatomical position (i.e. at right angle with the tibia) during the contractions. The maximum plantarflexion force for the training task was measured during the first training session of every week to determine the target force of 80% of the maximum force used in the training. Visual feedback of the instantaneous force was provided to the subjects during training.

Muscle-tendon unit properties

Lying prone with the hip and knee fully extended and the ankle joint in anatomical position, the gastrocnemius medialis muscle was imaged at the mid-belly using ultrasound with a transducer length of 60mm (LS 128 Telemed, Vilnius, Lithuania). Fascicle length, pennation angle and muscle thickness were analysed offline (ImageJ, National Institutes of Health, Bethesda, USA). Fascicle length was segmented manually, as the distance between the upper and lower aponeuroses along a direction parallel to the visible portions of fascicles. Pennation angle was defined as the orientation of fascicles relative to the deep aponeurosis.

In the same position, subjects were then securely strapped into a dynamometer and asked to perform isometric plantarflexions (IsoMed 2000 D&R Ferstl GmbH, Hemau, Germany). The highest torque of two attempts was set as the maximum torque. Subsequently, subjects were asked to perform ramp contractions, while ultrasound scans (LS 128 Telemed, Vilnius, Lithuania, 80 Hz) of the gastrocnemius myotendinous junction, plantarflexion torque (sampled at 600 Hz) and marker trajectories (Qualisys, Gothenburg, Sweden, sampled at 120 Hz) were recorded simultaneously to estimate tendon stiffness. Using visual feedback, subjects were instructed to increase torque at a loading rate of 100 N·m·s^{-1} up to 90 % of the individual maximum torque. An ultrasound gel pad was placed between the transducer and the skin to ensure consistent transmission of sound waves when the muscle was bulging. A bidirectional second order low-pass Butterworth filter with a cut-off frequency of 15 Hz was used to smooth the data.

Ultrasound images were analysed offline via semi-automatic tracking of a fascicle insertion as close as possible to the gastrocnemius myotendinous junction (Tracker 4.95, physlets.org/tracker/). A four-camera motion capture system was used to record the position of a triad of reflective markers, which were rigidly attached to the ultrasound probe. Using prior calibration, the position tracked in the two-dimensional ultrasound image was projected into the three-dimensional coordinate system of the laboratory. The position of the Achilles tendon insertion was determined with ultrasonography and identified externally with a reflective marker. Achilles tendon length was subsequently calculated as the straight distance between the myotendinous junction and the Achilles tendon insertion in the three-dimensional coordinate system of the laboratory.

The measured joint torque was corrected for unavoidable ankle joint rotation by recording the position of reflective markers on the medial malleolus and the footplate of the dynamometer (Arampatzis et al. 2005). The torque contribution of the triceps surae (91%) was estimated from normative data including the relative volume, optimal fibre length and moment arm of this muscle group (Dick et al. 2016, Supplementary Figure 3). Achilles tendon moment arm was estimated as the mean perpendicular distance between the line of action of the Achilles tendon and the mid-point of the mediolateral distance between the malleoli. Subsequently, Achilles tendon force was calculated by dividing the resultant torque by the Achilles tendon moment arm.

Tendon stiffness was obtained for every subject and test session from the mean force-elongation relationship of three trials. Five trials were recorded for this purpose and the trials yielding the lowest and highest stiffness values were excluded. The mean force-elongation curves were first fitted with a third-order polynomial and stiffness was calculated as the slope of the linear portion between 50% and 80% of the maximum individual force. All strain measurements were obtained for each individual at 80% of the force produced at the baseline test.

Muscle-tendon mechanics during running

Data were collected while the subjects were running at their preferred speed on one force plate of an instrumented treadmill (Force-Link, Motek, Netherlands, sampling at 1500 Hz). Force data were synchronized with three dimensional kinematic data recorded using at least 12 cameras of a motion capture system (Qualisys, Gothenburg, Sweden, sampled at 300 Hz). Raw data were filtered using a bidirectional second order low-pass Butterworth filter with a cut-off frequency of 15 Hz. Reflective markers were placed according to a modified Cleveland Clinics marker set to define pelvis, thigh, shank and foot segments and to track their movement during motion trials (left and right anterior and posterior iliac spine; right medial and lateral epicondyles; right medial and lateral malleoli; posterior calcaneus and first, second and fifth metatarsal; clusters of four markers to track the right thigh and shank segments). Ankle and knee joint angles and moments were calculated using inverse kinematics and kinetics analysis performed in Visual3D (C-Motion, Germantown, USA). The MTU length of gastrocnemius medialis and soleus was estimated using joint angle data and shank length defined as the distance from the lateral malleolus to the lateral epicondyle (Hawkins & Hull 1990).

During running, the ultrasound transducer was secured over the myotendinous junction or the muscle belly of the gastrocnemius using self-adhesive bandages. Ultrasound images were sampled at 80 Hz (60 mm, LS 128 Telemed, Vilnius, Lithuania). Achilles tendon length and Achilles tendon forces were estimated according to the same method as described for prone measurements. Software for the semi-automated tracking of fascicles in ultrasound images was used to analyse fascicle length and pennation angle during the running trials (Cronin et al. 2011; Farris & Lichtwark 2016). Subsequently, muscle thickness was estimated by multiplying fascicle length by the sine of pennation angle.

Data analysis and statistics

Fascicle and MTU length data were differentiated with respect to time to calculate their respective velocities. The influence of fascicle rotation on fascicle and muscle shortening was quantified using a modified version of the architectural gear ratio described in Brainerd & Azizi (2005). Architectural gear ratio was calculated, similarly to (Hollville et al. 2018), as the ratio between muscle length change (vertical projection of the fascicle length) and fascicle length change during the stance phase and will therefore be referred to as architectural gear ratio stance (AGRs) hereafter. Due to variability in duration of the ground contact, all data were resampled to an equal number of 101 data points during the stance phase. For each subject ten steps were initially included in the analysis. Individual steps were excluded when they varied from the rest of the steps determined by visual inspection so that data were averaged over eight steps for the final analysis for each subject. Due to insufficient image quality, ultrasound data on soleus fascicles for three subjects of the training group were discarded and data on gastrocnemius myotendinous junction were discarded for two subjects of the training group and three controls.

Length changes, peak length and peak velocity values of the relevant variables were compared with a two-way ANOVA design using the factors time (pre vs. post training) and group (training vs. control group) for unloaded and loaded running. Bonferroni post-hoc tests were used where significant interaction or main effects were detected (Prism, GraphPad Software Inc., La Jolla, CA, USA). All variables were analysed during the stance phase. Statistical significance was set to $P < 0.05$.

Results

Muscle tendon properties and strength

The results for muscle-tendon properties and strength have been reported in detail in a previous study (Werkhausen et al. 2018) and are presented in Table 1. While isometric 1RM torque increased by 39% between the first and the last training week, the maximal torque measured in the dynamometer increased by 15% in the training group. Tendon stiffness increased by 18%, although tendon strain did not decrease significantly.

Table 1. *Plantarflexion torque, muscle architecture and Achilles tendon (AT) stiffness in the training group and the control group measured before (pre) and after (post) the training intervention (Werkhausen et al. 2018).*

	Training group		Control group	
	Pre	Post	Pre	Post
Torque [N m] ^{b}	172 ± 50	198 ± 51 ^{c}	170 ± 51	180 ± 62
Lf GM [mm]	892 ± 133	907 ± 161	842 ± 161	840 ± 104
PA GM [°]	18.1 ± 1.8	19.0 ± 2.0 ^{c}	18.4 ± 1.1	18.3 ± 1.7
Thickness GM [mm] ^{b}	237 ± 35	250 ± 38 ^{c}	231 ± 38	235 ± 36
AT stiffness [N mm-1] ^{a,b}	397 ± 146	459 ± 147 ^{c}	399 ± 193	400 ± 212
AT strain [mm]	4.4 ± 1.1	4.1 ± 0.5	4.0 ± 1.5	3.9 ± 1.5

Values are means ± sd; GM, gastrocnemius medialis; SOL, soleus; Lf, length of fascicle; PA, pennation angle; ^{a} $P < 0.05$ interaction effect; ^{b} $P < 0.05$ main effect of time; ^{c} $P < 0.05$ comparing pre- and post-intervention test.

Effect of training on running kinematics and kinetics

Running kinematics and kinetics were statistically similar pre and post training for subjects in both groups. A non-significant increase in maximum dorsiflexion (pre 34 ± 12°, post 40 ± 10°; *P* = 0.08) and subsequent maximum plantarflexion (pre 34 ± 4°, post 35 ± 7°; *P* = 0.21) was observed in the training group, whereas knee range of motion remained similar after training for maximum flexion (pre 26 ± 2°, post 27 ± 2°; *P* = 0.16) and maximum extension (pre 26 ± 5°, post 25 ± 4°; *P* = 0.99) during the stance phase. No significant differences were found for peak moments of the ankle joint (pre 206 ± 70 Nm, post 197 ± 57 Nm; *P* = 0.77) or knee joint (pre 126 ± 36 Nm, post 122 ± 48°; *P* = 0.93). Ankle and knee joint angles and moments during the stance phase of unloaded running are presented in **Figure 1** for the training group (**A-B**) and the control group (**C-D**) before and after training.

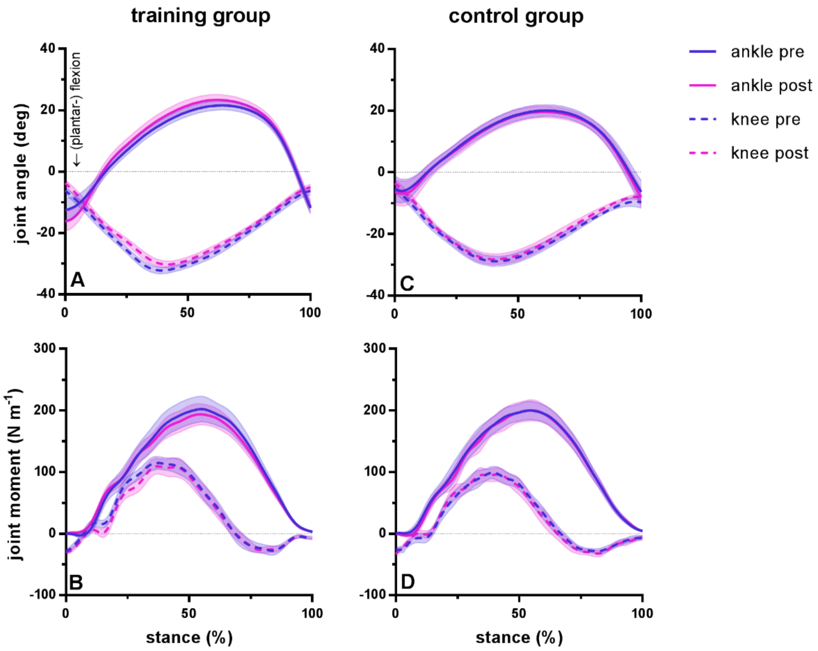

Figure 1. *Group mean ankle and knee joint angles and moments during the stance phase of barefoot running at preferred speed for the training group (**A-B**) and the control group (**C-D**). Time series are normalized to 101 points.*

Effect of training on muscle-tendon interaction during running

Figure 2 shows MTU, muscle fascicle and Achilles tendon length during the stance phase of running pre and post training for the training group (**A-C**) and the control group (**D-F**). The typical MTU stretch-shortening pattern during running did not differ in amplitude after training (**Figure 2A & D**) in the gastrocnemius (Interaction P = 0.11 and P = 0.25 for stretch and shortening, respectively) or the soleus (Interaction P = 0.11 and P = 0.76 for stretch and shortening, respectively). Muscle fascicles shortened throughout the stance phase but neither shortening amplitude nor peak fascicle velocity differed after training in either muscle (**Table 2, Figure 2B & E, Figure 3C & D**). However, the rotation of fascicles about their deeper insertion during the stance phase, i.e. the change in pennation angle, tended to increase in gastrocnemius (Interaction P = 0.10, Time P = 0.04, Post-test P = 0.01) but not soleus (non-significant interaction or time effect). Accordingly, there was a main effect of time for architectural gear ratio of gastrocnemius (P = 0.05), and post-hoc tests indicated a significant increase of this parameter in the trained group only (P = 0.01). There was no main effect of time for soleus (**Figure 4**). Similarly to the MTU, no difference was observed after training for Achilles tendon stretch (Interaction P = 0.69). However, we found an interaction effect for Achilles tendon shortening (P = 0.02). Post-hoc comparisons showed that tendon recoil was reduced by 30% in the training group ($P < 0.01$) whereas this variable did not change in the controls (**Table 2, Figure 2C, Figure 3B)**.

Table 2. *Relevant variables for muscle-tendon unit (MTU), Achilles tendon (AT) and fascicles (F) of gastrocnemius (GM) and soleus (SOL) behaviour during the stance phase of running at preferred speed. Data are presented for the training group and the control group before (pre) and after (post) the training intervention designed to increase tendon stiffness.*

		training group			**control group**		
		pre	**post**	**diff**	**pre**	**post**	**diff**
Lengthening (mm)	**MTU GM**	26 ± 10	30 ± 9	5 ± 7	19 ± 7	19 ± 7	0 ± 2
	MTU SOL	26 ± 10	32 ± 9	6 ± 8	21 ± 7	22 ± 7	0 ± 2
	AT	8 ± 3	8 ± 2	0 ± 4	10 ± 3	11 ± 4	1 ± 2
Shortening (mm)	**MTU GM**	26 ± 4	27 ± 7	1 ± 4	21 ± 8	22 ± 7	1 ± 3
	MTU SOL	27 ± 4	29 ± 8	2 ± 5	22 ± 9	23 ± 7	1 ± 3
	AT [a]	13 ± 2	9 ± 4 [c]	4 ± 4	10 ± 3	10 ± 3	1 ± 2
	F GM	18 ± 5	20 ± 2	2 ± 5	19 ± 5	20 ± 4	1 ± 4
	F SOL	10 ± 3	11 ± 3	1 ± 2	8 ± 3	10 ± 2	2 ± 3
Shortening vel (mm s^{-1})	**F GM**	179 ± 51	205 ± 41	26 ± 50	163 ± 48	166 ± 17	3 ± 41
	F SOL	108 ± 41	133 ± 41	25 ± 40	95 ± 22	114 ± 17	19 ± 25
AGRs	**GM** [b]	1.06 ± 0.03	1.09 ± 0.03 [c]	0.03 ± 0.04	1.07 ± 0.03	1.08 ± 0.02	0.0 ± 0.02
	SOL	1.06 ± 0.11	1.05 ± 0.11	0.01 ± 0.08	1.12 ± 0.09	1.15 ± 0.09	0.03 ± 0.07
Change in pennation (°)	**GM** [a,b]	7.7 ± 3.4	10.2 ± 2.2 [c]	2.5 ± 2.7	9.4 ± 3.2	9.7 ± 2.0	0.3 ± 2.5
	SOL	8.8 ± 2.8	9.8 ± 4.1	1.0 ± 3.1	9.6 ± 3.1	12.6 ± 3.8	2.9 ± 4.4
Thickness change (mm)	**GM**	0.8 ± 0.9	0.6 ± 1.0	0.1 ± 1.1	0.7 ± 0.7	0.6 ± 0.6	0.1 ± 0.7
	SOL	3.2 ± 2.9	3.7 ± 2.2	0.5 ± 1.4	2.5±1.0	3.1 ± 1.4	0.5 ± 1.4
Force (N)	**AT**	4315 ± 1646	4041 ± 1331	274 ± 1142	3632 ± 765	3765 ± 917	133 ± 279

Values are means ± sd.; [a] $P < 0.05$ interaction effect; [b] $P < 0.05$ main effect of time; [c] $P < 0.05$ comparing pre- and post-intervention test; diff, absolute difference between pre- and post- intervention test. Architectural gear ratio during stance (AGRs) was calculated as the ratio between fascicle length change and muscle length change during the stance phase.

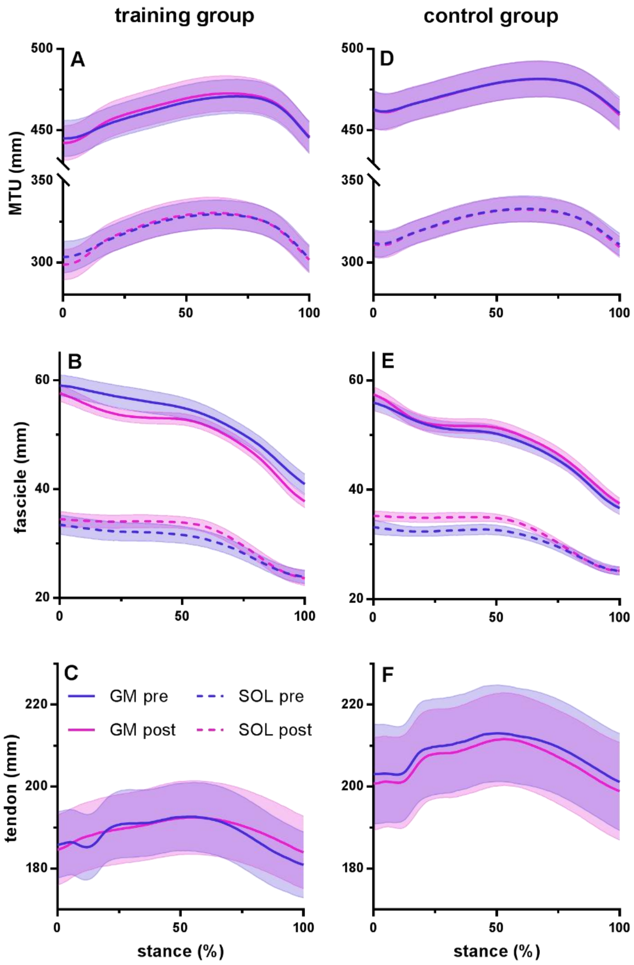

Figure 2. *Muscle-tendon unit (MTU), fascicle and tendon length in the training group (**A-C**) and the control group (**D-F**) for gastrocnemius medialis (GM) and soleus (SOL) before (pre) and after (post) the training during the stance phase of running. Time series are normalized to 101 points. Values are means ± s.e.m..*

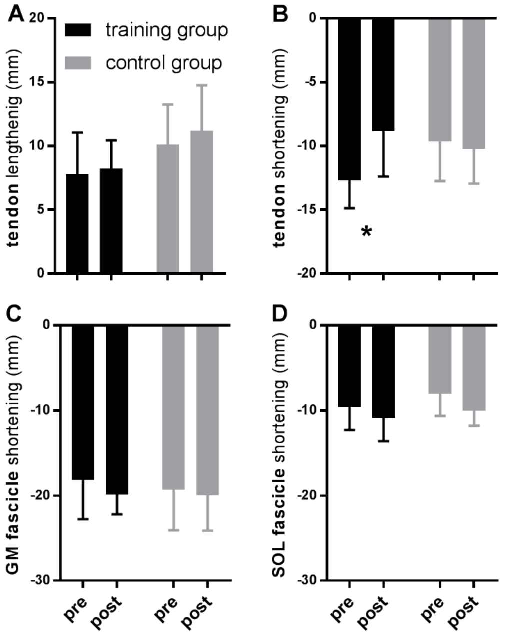

*__Figure 3__. Tendon (__A-B__) and fascicle (__C-D__) measures during the stance phase of running compared between tests pre- and post-training in the training (black) and the control group (grey). Data are means ± s.d., * P < 0.05 when comparing pre vs. post intervention.*

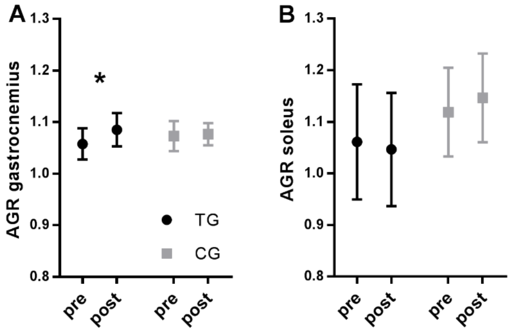

*__Figure 4.__ Architectural gear ratio during the stance phase of running (AGRs) for gastrocnemius medialis (__A__) and soleus (__B__) compared between tests pre- and post-training in the training (black) and the control group (grey). AGRs was calculated as the ratio of muscle strain and fascicle strain during the stance phase. Data are means ± s.d., * P < 0.05 when comparing pre vs. post intervention.*

Discussion

The present study examined the effect of a training-induced increase in Achilles tendon stiffness on muscle-tendon mechanics during running. After the 10-week training intervention, isometric MVC of the plantarflexor muscles increased by 15% and Achilles tendon stiffness increased by 18%. Gastrocnemius muscle thickness and pennation angle increased (5% each) but fascicle length remained unchanged. Contrary to our predictions, tendon stretch during stance was not reduced after training but tendon recoil was reduced. In accordance with previous findings (Lai et al. 2018), fascicles of the monoarticular soleus muscle displayed a lower shortening velocity than the gastrocnemius in the first part of the stance phase. Neither muscle seemed to present different fascicle lengthening patterns after training, although altered muscle mechanics during running stance were indicated by an increase in gastrocnemius AGRs after the intervention. None of the examined parameters changed in the control group, supporting the notion that these findings resulted from training adaptations.

Effect of training on muscle-tendon properties

The effect of training on muscle architecture and tendon stiffness is discussed in greater detail elsewhere (Werkhausen et al. 2018). Overall, the intervention successfully increased the stiffness of the Achilles tendon while avoiding changes in resting fascicle length. The latter was of particular importance to verify the hypothesized influence of in series elasticity on fascicle behaviour. The 18% increase in Achilles tendon stiffness was within the range reported in other studies (Wiesinger et al. 2015). Bearing in mind the short time under tension during exercise (total of 40 sec per session), with the intent to limit adaptations in muscle architecture, greater increases in tendon stiffness seem likely with larger exercise loads. We can nonetheless speculate that the observed stiffening of the whole Achilles tendon would apply to the so-called free tendon and possibly to other collagenous structures, such as aponeuroses. Surprisingly, tendon strain during isometric contractions was not reduced significantly after training, which may be a consequence of the relatively low force produced during this test, or the lack of sensitivity of *in vivo* tendon strain and slack length measurements (Seynnes et al. 2015). The lack of strain reduction could also result from changes in the toe-region of the force-elongation relationship of the tendon. The other main effect of the training intervention was the increase in plantarflexion force and associated hypertrophy, as indicated by small increases in gastrocnemius pennation angle and thickness. The increase in force production capacity was unavoidable with this type of training and is unlikely to have substantially influenced fascicle behaviour during the running test (see below).

Similar tendon strain but reduced recoil during running

Consistent with isometric tests but contrary to our hypothesis, the tendon stretch occurring during the first part of the stance phase was not reduced after training, in spite of the greater stiffness. The same methodological limitations mentioned for the isometric tests (i.e. measurement sensitivity) may contribute to explaining this result. However, all other parameters being equal, the estimated tendon forces produced under the present running conditions would be expected to reduce tendon elongation by about 19% after training (estimation based ratios of mean tendon force to mean tendon stiffness of the training group, before and after training). With a coefficient of variation of 10% (calculated from the control group data), the sensitivity of the method used to calculate tendon length during running is deemed sufficient to detect such a reduction in tendon elongation. Additionally, estimates of tendon length per se, as used in this study, likely provide better estimates of tendon energy storage than indirect methods reflecting the whole SEE (Zelik & Franz 2017). Yet, in agreement with our results, Albracht & Arampatzis (2013) previously found no change in SEE strain, which they attributed to increased forces and joint moments. However, in our study, GRF and ankle and knee joint moments were similar after training, suggesting that the force applied to the Achilles tendon during stance did not increase. An alternative explanation for the unchanged longitudinal stretch of the tendon could be related to the deformation pattern of the aponeuroses. Biaxial strains have been shown to occur in the proximal part of the human Achilles tendon and the aponeurosis, and to influence the longitudinal stiffness of aponeuroses in animals (Azizi & Roberts 2009). Biaxial loading may have the same effect upon the sheet-like proximal part of the tendon from which they arise. Hence, a change in muscle contractile behaviour (discussed below) and in transverse strain of the aponeurosis and the proximal region of the Achilles tendon may have reduced the longitudinal stiffness of these structures, resulting in a similar stretch of the whole tendon after training.

In contrast with its elongation, the recoil of the Achilles tendon during the late stance phase was reduced after training. The reduction in tendon recoil alone would suggest a lower elastic energy return during push-off but we expect the greater tendon stiffness to have in part compensated this effect. In fact, the similar joint moments measured during this phase after training are compatible with a similar elastic energy return.

Similar fascicle shortening but increased gearing in gastrocnemius during running

Contrary to predictions based on two-dimensional models of muscle-tendon units, we did not observe any post-training difference in changes in gastrocnemius or soleus fascicle length and velocity during stance. This result is in line with a previous study, which did not find differences in gastrocnemius fascicle shortening pattern during running after 14 weeks of isometric plantarflexion training (Albracht & Arampatzis 2013).

However, we found that contractile behaviour of the gastrocnemius muscle was altered, as evidenced by the increased change in pennation angle during stance after the training intervention. Although the methods used in the present study did not allow a precise estimation of instantaneous architectural gear ratio (the ratio of muscle velocity and fascicle velocity), our calculation of muscle lengthening relative to fascicle lengthening during stance phase was also increased after training. These results suggest that, although gastrocnemius fascicle shortening patterns were preserved, the contraction behaviour of the gastrocnemius muscle during stance did not remain completely identical. Such changes in contractile patterns are to our knowledge reported for the first time after a training intervention and should only be interpreted with caution, since muscle variable gearing has thus far only been studied in cross-sectional comparisons. Differences in AGR have typically been linked to force levels (Azizi et al. 2008; Dick & Wakeling 2017) or by extension, to the stiffness of the connective tissue placed in series with muscle fascicles (Eng & Roberts 2018). A possible explanation for the present changes in fascicle angular excursion and in AGRs after training may simply be geometric, as a result of the increases in resting pennation angle and muscle thickness. On the other hand, the training intervention may also have stiffened the aponeurosis and tendon radially (see discussion above). In vitro data have shown, albeit with a drastic protocol consisting of aponeurosis incisions, the influence of radial stiffness on gearing at relatively high forces (Eng & Roberts 2018). Hence, the altered AGRs found in the present study could also be connected to changes in radial stiffness of the aponeurosis and/or the proximal part of the Achilles tendon. However, greater muscle bulging consistent with a higher AGRs was not observed here, based on our calculations of muscle thickness. Unfortunately, determining muscle thickness using trigonometry (assuming parallel aponeuroses) and the compression caused by the strapping of the ultrasound transducer may have hampered the sensitivity of this approach, so this variable may not be reliable. Considering both changes in Achilles tendon strain pattern and gastrocnemius contractile pattern, a training induced change in architectural gear ratio appears to be the most likely mechanistic hypothesis.

Interestingly, our data show that the soleus fascicle shortening pattern also did not change after training. Methodological difficulties prevented us from measuring the relative strains of the whole Achilles tendon and its free portion but previous authors have shown that SEE of the soleus undergoes a greater stretch than that of the gastrocnemii during stance (Lai et al. 2018). This is consistent with measurements of tendon displacement during isometric plantarflexions at different joint angles (Clark & Franz 2018). Yet the hypothesised greater sensitivity of soleus fascicle lengthening pattern to changes in tendon stiffness was not seen in the present study. An insufficient increase in stiffness of the free tendon to reduce strain appreciably after training could explain the unchanged soleus fascicle shortening. The effect of resistance training on the free tendon has to our knowledge never been reported and further research is required to assess the effects of training on stiffness and strain of the free tendon specifically. Alternatively, changes in bi-axial loading of the soleus aponeurosis may also have occurred after training, preserving the contractile conditions of the fascicles and the important force production capacity of this muscle. However, the lack of change in soleus AGRs refutes this interpretation, incidentally suggesting that triceps surae muscles are not affected similarly by training.

Methodological considerations

There are limitations inherent to the present methodology that should be acknowledged. To decrease the noise of the ultrasound data in a complex dynamic task such as running, we chose to use a relatively simple quantification of the architectural gear ratio. As originally proposed by Brainerd & Azizi (2005), the strain of muscle and fascicle were used to calculate the ratio, whereas we defined muscle strain as the vertical projection of the fascicle length change. Although the development of the architectural gear ratio over time is admittedly not considered with this approach, the index provides information about the relationship of muscle to fascicle length changes during the stance phase. Because fascicles shorten during the whole stance phase, we believe that this ratio appropriately quantifies fascicle shortening in relation to muscle shortening.

Conclusion

In line with model-based predictions, this study showed that the *in vivo* behaviour of the Achilles tendon during running was altered following a training intervention aimed at increasing its stiffness. Changes in kinematics of tendon stretch and recoil differed after training, which may be attributable to differences in the way energy was stored and released. Yet these changes did not affect fascicle lengthening patterns in either the bi-articular gastrocnemius or the mono-articular soleus, despite expected differences in the sensitivity of these muscles to changes in tendon mechanical properties. However, increased gastrocnemius gear ratio during the stance phase demonstrated that the contractile behaviour of this muscle was altered as a result of the intervention. We suggest that training adaptations alter muscle-tendon interaction in more complex ways than suggested by in series models, via active regulation of tendon-aponeuroses stiffness and different adjustments in the behaviour of synergistic muscles.

Acknowledgements

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References

Albracht K, and Arampatzis A. 2013. Exercise-induced changes in triceps surae tendon stiffness and muscle strength affect running economy in humans. *European Journal of Applied Physiology* 113:1605-1615. 10.1007/s00421-012-2585-4

Arampatzis A, Karamanidis K, Morey-Klapsing G, De Monte G, and Stafilidis S. 2007. Mechanical properties of the triceps surae tendon and aponeurosis in relation to intensity of sport activity. *Journal of Biomechanics* 40:1946-1952. 10.1016/j.jbiomech.2006.09.005

Arampatzis A, Morey-Klapsing G, Karamanidis K, DeMonte G, Stafilidis S, and Brüggemann G-P. 2005. Differences between measured and resultant joint moments during isometric contractions at the ankle joint. *Journal of Biomechanics* 38:885-892. 10.1016/j.jbiomech.2004.04.027

Azizi E, Brainerd EL, and Roberts TJ. 2008. Variable gearing in pennate muscles. *Proceedings of the National Academy of Sciences* 105:1745-1750. 10.1073/pnas.0709212105

Azizi E, and Roberts TJ. 2009. Biaxial strain and variable stiffness in aponeuroses. *The Journal of Physiology* 587:4309-4318. 10.1113/jphysiol.2009.173690

Brainerd EL, and Azizi E. 2005. Muscle fiber angle, segment bulging and architectural gear ratio in segmented musculature. *Journal of Experimental Biology* 208:3249. 10.1242/jeb.01770

Clark WH, and Franz JR. 2018. Do triceps surae muscle dynamics govern non-uniform Achilles tendon deformations? *PeerJ* 6:e5182. 10.7717/peerj.5182

Cronin NJ, Carty CP, Barrett RS, and Lichtwark G. 2011. Automatic tracking of medial gastrocnemius fascicle length during human locomotion. *Journal of Applied Physiology* 111:1491-1496. 10.1152/japplphysiol.00530.2011

Dick TJM, Arnold AS, and Wakeling JM. 2016. Quantifying Achilles tendon force in vivo from ultrasound images. *Journal of Biomechanics* 49:3200-3207. 10.1016/j.jbiomech.2016.07.036

Dick TJM, and Wakeling JM. 2017. Shifting gears: dynamic muscle shape changes and force-velocity behaviour in the medial gastrocnemius. *Journal of Applied Physiology* 123:1433-1442. 10.1152/japplphysiol.01050.2016

Eng CM, and Roberts TJ. 2018. Aponeurosis influences the relationship between muscle gearing and force. *Journal of Applied Physiology*. 10.1152/japplphysiol.00151.2018

Farris DJ, and Lichtwark GA. 2016. UltraTrack: Software for semi-automated tracking of muscle fascicles in sequences of B-mode ultrasound images. *Computer Methods and Programs in Biomedicine* 128:111-118. 10.1016/j.cmpb.2016.02.016

Hawkins D, and Hull ML. 1990. A method for determining lower extremity muscle-tendon lengths during flexion/extension movements. *Journal of Biomechanics* 23:487-494. 10.1016/0021-9290(90)90304-L

Hollville E, Nordez A, Guilhem G, Lecompte J, and Rabita G. 2018. Interactions between fascicles and tendinous tissues in gastrocnemius medialis and vastus lateralis during drop landing. *Scandinavian Journal of Medicine and Science in Sports* 0. 10.1111/sms.13308

Kubo K, Miyazaki D, Shimoju S, and Tsunoda N. 2015. Relationship between elastic properties of tendon structures and performance in long distance runners. *European Journal of Applied Physiology* 115:1725-1733. 10.1007/s00421-015-3156-2

Lai AKM, Lichtwark GA, Schache AG, and Pandy MG. 2018. Differences in in vivo muscle fascicle and tendinous tissue behavior between the ankle plantarflexors during running. *Scandinavian Journal of Medicine and Science in Sports* 28:1828-1836. 10.1111/sms.13089

Lichtwark GA, and Wilson AM. 2007. Is Achilles tendon compliance optimised for maximum muscle efficiency during locomotion? *Journal of Biomechanics* 40:1768-1775. 10.1016/j.jbiomech.2006.07.025

Lichtwark GA, and Wilson AM. 2008. Optimal muscle fascicle length and tendon stiffness for maximising gastrocnemius efficiency during human walking and running. *Journal of Theoretical Biology* 252:662-673. 10.1016/j.jtbi.2008.01.018

McCrum C, Leow P, Epro G, König M, Meijer K, and Karamanidis K. 2018. Alterations in Leg Extensor Muscle-Tendon Unit Biomechanical Properties With Ageing and Mechanical Loading. *Frontiers in Physiology* 9:150. 10.3389/fphys.2018.00150

Obst SJ, Heales LJ, Schrader BL, Davis SA, Dodd KA, Holzberger CJ, Beavis LB, and Barrett RS. 2018. Are the Mechanical or Material Properties of the Achilles and Patellar Tendons Altered in Tendinopathy? A Systematic Review with Meta-analysis. *Sports Medicine* 48:1-20. 10.1007/s40279-018-0956-7

Roberts TJ, Marsh RL, Weyand PG, and Taylor CR. 1997. Muscular Force in Running Turkeys: The Economy of Minimizing Work. *Science* 275:1113-1115. 10.1126/science.275.5303.1113

Seynnes OR, Bojsen-Møller J, Albracht K, Arndt A, Cronin NJ, Finni T, and Magnusson SP. 2015. Ultrasound-Based Testing Of Tendon Mechanical Properties: A Critical Evaluation. *Journal of Applied Physiology* 18:133-141. 10.1152/japplphysiol.00849.2014

Werkhausen A, Albracht K, Cronin NJ, Paulsen G, Bojsen-Møller J, and Seynnes OR. 2018. Effect of Training-Induced Changes in Achilles Tendon Stiffness on Muscle–Tendon Behavior During Landing. *Frontiers in Physiology* 9:794. 10.3389/fphys.2018.00794

Wiesinger H-P, Kösters A, Müller E, and Seynnes OR. 2015. Effects of Increased Loading on In Vivo Tendon Properties: A Systematic Review. *Medicine and Science in Sports and Exercise* 47:1885-1895. 10.1249/mss.0000000000000603

Zelik KE, and Franz JR. 2017. It's positive to be negative: Achilles tendon work loops during human locomotion. *PloS One* 12:e0179976. 10.1371/journal.pone.0179976

Appendix 1

Approval letter from the Ethical Committee

Olivier Seyness
Seksjon for fysisk prestasjon

OSLO 28. august 2017

Søknad 14-220817 – Rollen av muskel- og sene-egenskaper i den mekaniske funksjon av de menneskelige nedre ekstremitetene

Vi viser til søknad, prosjektbeskrivelse og innsendt og godkjent søknad til NSD.

I henhold til retningslinjer for behandling av søknad til etisk komite for idrettsvitenskapelig forskning på mennesker, ble det i komiteens møte av 22. august 2017 konkludert med følgende:

Vedtak

På bakgrunn av forelagte dokumentasjon finner komiteen at prosjektet er forsvarlig og at det kan gjennomføres innenfor rammene av anerkjente etiske forskningsetiske normer nedfelt i NIHs retningslinjer. Til vedtaket har komiteen lagt følgende forutsetning til grunn:

- *At eventuelle vilkår fra NSD følges*

Komiteen gjør videre oppmerksom på at vedtaket er avgrenset i tråd med fremlagte dokumentasjon. Dersom det gjøres vesentlige endringer i prosjektet som kan ha betydning for deltakernes helse og sikkerhet, skal dette legges fram for komiteen før eventuelle endringer kan iverksettes.

For fremtidige søknader, så anbefaler komiteen at informasjonsskriv til forskningsdeltakere utarbeides i henhold til REK mal.

Med vennlig hilsen
Professor Sigmund Loland
Leder, Etisk komite, Norges idrettshøgskole

Appendix 2

Approval letter from the Norwegian Centre for Research Data

Amelie Werkhausen
Seksjon for fysisk prestasjonsevne Norges idrettshøgskole
Postboks 4014
0806 OSLO

Vår dato: 13.06.2017	Vår ref: 54377 / 3 / STM	Deres dato:	Deres ref:

TILBAKEMELDING PÅ MELDING OM BEHANDLING AV PERSONOPPLYSNINGER

Vi viser til melding om behandling av personopplysninger, mottatt 09.05.2017. Meldingen gjelder prosjektet:

54377	*Rollen av muskel- og sene-egenskaper i den mekaniske funksjon av de menneskelige nedre ekstremitetene*
Behandlingsansvarlig	*Norges idrettshøgskole, ved institusjonens øverste leder*
Daglig ansvarlig	*Amelie Werkhausen*

Personvernombudet har vurdert prosjektet, og finner at behandlingen av personopplysninger vil være regulert av § 7-27 i personopplysningsforskriften. Personvernombudet tilrår at prosjektet gjennomføres.

Personvernombudets tilråding forutsetter at prosjektet gjennomføres i tråd med opplysningene gitt i meldeskjemaet, korrespondanse med ombudet, ombudets kommentarer samt personopplysningsloven og helseregisterloven med forskrifter. Behandlingen av personopplysninger kan settes i gang.

Det gjøres oppmerksom på at det skal gis ny melding dersom behandlingen endres i forhold til de opplysninger som ligger til grunn for personvernombudets vurdering. Endringsmeldinger gis via et eget skjema, http://www.nsd.uib.no/personvernombud/meld_prosjekt/meld_endringer.html. Det skal også gis melding etter tre år dersom prosjektet fortsatt pågår. Meldinger skal skje skriftlig til ombudet.

Personvernombudet har lagt ut opplysninger om prosjektet i en offentlig database, http://pvo.nsd.no/prosjekt.

Personvernombudet vil ved prosjektets avslutning, 01.10.2018, rette en henvendelse angående status for behandlingen av personopplysninger.

Vennlig hilsen

Kjersti Haugstvedt

Siri Tenden Myklebust

Kontaktperson: Siri Tenden Myklebust tlf: 55 58 22 68
Vedlegg: Prosjektvurdering

Dokumentet er elektronisk produsert og godkjent ved NSDs rutiner for elektronisk godkjenning.

Prosjektvurdering - Kommentar

Prosjektnr: 54377

FORMÅL
Prosjektet har som mål å undersøke påvirkningen av kroniske tilpasninger av akillessenen til forskjellige belastninger. Hovedmålet er å undersøke rollene av distinkte sene egenskaper på muskel-sene enheten i funksjonelle bevegelser med forskjellige energetiske krav.

REK har vurdert at prosjektet faller utenfor helseforskningslovens virkeområde (2016/338/REK sør-øst A).

UTVALG OG REKRUTTERING
Utvalget består av friske personer mellom 18 og 35 år. Deltakerne rekrutteres via annonser/oppslag.

DATA
Data samles inn ved medisinske undersøkelser/tester. Det vil samles inn antropometriske og fysiologiske data som høyde, vekt, segmentlengder av leggen, muskel fascikle og senens lengder. Dessuten skal kinetisk og kinematisk informasjonen bli innsamlet (reaksjonskrefter fra underlaget, leddvinkler).

Vi finner at det vil behandles sensitive personopplysninger om helseforhold.

INFORMASJON OG SAMTYKKE
Utvalget informeres skriftlig og muntlig om prosjektet og samtykker til deltakelse. Informasjonsskrivet er godt utformet, men vi ber om at følgende tas bort før utvalget kontaktes:
- Formuleringen «[…]Dette vil ikke få konsekvenser for din videre behandling (Siste setning fjernes dersom deltakeren ikke rekrutteres i kraft av å være pasient).» tas bort.
- Videre bør henvisningen til innsamlede prøver tas bort.
- Setningen om at data anonymiseres eller slettes fem år etter prosjektslutt tas bort. I stedet må det opplyses om at innsamlede opplysninger slettes/anonymiseres ved prosjektets avslutning. Vi viser her til epost fra forsker, mottatt 13.06.2017.

DATASIKKERHET
Personvernombudet legger til grunn at forsker etterfølger Norges idrettshøgskole sine interne rutiner for datasikkerhet. Dersom personopplysninger skal lagres på mobile enheter, bør opplysningene krypteres tilstrekkelig.

PROSJEKTSLUTT OG ANONYMISERING
Forventet prosjektslutt er 01.10.2018. Ifølge prosjektmeldingen skal innsamlede opplysninger da anonymiseres. Anonymisering innebærer å bearbeide datamaterialet slik at ingen enkeltpersoner kan gjenkjennes. Det gjøres ved å:
- slette direkte personopplysninger (som navn/koblingsnøkkel)

- slette/omskrive indirekte personopplysninger (identifiserende sammenstilling av bakgrunnsopplysninger som f.eks. bosted/arbeidssted, alder og kjønn)

Appendix 3

Summary in German

Kurzfassung (Abstract in German)

Die Leistung der Muskel-Sehnen-Einheit (MSE) ist abhängig von der viskoelastischen Beschaffenheit des Sehnengewebes. Bei den verschiedensten Bewegungen schafft die Sehne vorteilhafte Kontraktionsbedingungen für Muskeln. Dennoch ist unser Wissen hinsichtlich der Modulation von Muskel-Sehnen-Verhalten unter verschiedenen Bedingungen und mit unterschiedlicher Sehnensteifigkeit unzureichend. Neben generellen Lücken im Forschungsstand wurde am Menschen in der Vergangenheit die Rolle von Sehnen zudem hauptsächlich im Zusammenhang mit Energiespeicherung und Leistungsverstärkung untersucht und nicht bei Bewegungen, die Energiedissipation erfordern.Das Ziel dieser Arbeit war mithin die Untersuchung der Interaktion von Achillessehne und der Muskeln gastrocnemius und soleus unter den folgenden Bedingungen: während einer Landungsbewegung, unter äußeren Einschränkungen (zusätzliche Last und Laufgeschwindigkeit) bei Landungen und beim Laufen, und mit trainingsinduzierter Erhöhung der Sehnensteifigkeit bei Landungen und beim Laufen.

Durchgeführt wurden eine Querschnitts- (A) und eine Längsschnittstudie (B), um Muskel- und Sehnenverhalten bei Landungen (n = 39 und n = 21) und beim Laufen (n=16 und n=2) zu erforschen. In Studie A wurden bei beiden Aufgaben Fuß- und Kniegelenkmechanik sowie gastrocnemius und soleus MSE-Länge mit Hilfe kinematischer und kinetischer Daten bestimmt. Diese Daten wurden zudem mit Ultraschall Messungen synchronisiert, um Achillessehnen- und Muskelfaszikellängen zu bestimmen. Nachdem beide Aufgaben ohne Zusatzlast bewältigt wurden, wiederholten die Probanden die Aufgaben mit Gewichtswesten, die eine Masse von 20% ihres Körpergewichts hatten. Laufgeschwindigkeit in beiden Lastszenarien war einerseits eine individuell gewählte bevorzugte Geschwindigkeit und andererseits eine Geschwindigkeit, die 20% schneller als die individuell gewählte war. In Studie B führte die Trainingsgruppe (n = 11) ein 10 wöchiges Trainingsprogramm bestehend aus einbeinigen isometrischen plantarflexions Kontraktionen durch. Studie B folgte einem ähnlichen Testprotokoll wie Studie A (ohne die experimentellen Bedingungen von Zusatzlast und erhöhter Geschwindigkeit), jedoch wurden zusätzlich Messungen von Plantarflexionskraft und Sehnensteifigkeit bei isometrischen Kontraktionen inkludiert. Unterschiede zwischen den verschiedenen Testbedingungen (Studie A) bzw. zwischen Pre- und Postinterventionstest (Studie B) in den relevanten Variablen wurden mit ANOVAs mit wiederholten Messungen getestet.

Die Landungsaufgabe in Studie A zeigte, dass die schnelle Verlängerung der MSE von der Achillessehne aufgenommen wurde, während gastrocnemius und soleus Faszikel sich mit geringeren Geschwindigkeiten als die gesamte MSE verlängerten. Das Tragen der Zusatzlast führte zu einer größeren Dehnung der Sehne und die zusätzliche negative Arbeit wurde von höherer soleus Muskelaktivität geleistet, während die Verlängerung der Faszikel unverändert blieb. Beim Laufen führte die zusätzliche Last zu einem vergrößerten Rückschlag der Achillessehne, wobei die Dehnung der Sehne unverändert blieb. Demgegenüber resultierte die höhere Geschwindigkeit in erhöhter Muskelaktivität von gastrocnemius und soleus. Sowohl Faszikellänge als auch -geschwindigkeit beider Muskeln blieben unverändert beim Laufen mit Zusatzlast und erhöhter Geschwindigkeit. In Studie B war eine um 18% erhöhte Steifigkeit der Achillessehne nach Abschluss des Trainingsprogramms gemessen worden. Ein gleichzeitiger Anstieg der platarflexions Kraft um 15% war begleitet von einer Vergrößerung des Muskelfiederungswinkels und einer Erhöhung der Muskeldicke (um jeweils 5%, gemessen in Ruhe) in der Trainingsgruppe, wobei die Länge der Faszikel unverändert blieb. Die Landungsaufgabe resultierte, trotz unveränderter Sehnenkraft und erhöhter Sehnensteifigkeit, nicht in einer messbaren Änderung der Dehnung der Achillessehne. Die Verlängerung und die Geschwindigkeit der Faszikel waren nach der Trainingsintervention verringert (um 27 bzw. 21%) und erstaunlicherweise waren gastrocnemius Faszikel zu Beginn der Landung um 8% länger. Für soleus wurden zwischen den Tests keine Unterschiede festgestellt. Beim Laufen blieb die Dehnung der Sehne unverändert durch das Trainingsprogramm, wobei der Rückschlag um 30% reduziert war. Das Training hatte zudem keine Auswirkungen auf die Kraft in der Achillessehne und auf die Verkürzung von gastrocnemius und soleus Faszikeln beim Laufen. Dennoch wird durch die erhöhte Änderung des Fiederungswinkels und durch das erhöhte architektonische Übersetzungsverhältnis während der Standphase eine Veränderung der gastrocnemius Muskelmechanik suggeriert. Keine der Variablen änderte sich in der Kontrollgruppe nach dem Trainingszeitraum.

Diese Resultate zeigen, dass bei Landungen die schnelle Verlängerung der MSE von der Achillessehne abgepuffert wurde, was zugleich die Verlängerung der Faszikel begrenzte. Die erhöhte Muskelaktivtat ermöglichte die Aufnahme der zusätzlichen Last durch die Sehne. Dies stellt einen wirkungsvollen Mechanismus dar, Faszikellänge und -geschwindigkeit beibehalten zu können. Das Kontraktionsverhalten der Faszikel war auch beim Laufen mit Last und bei erhöhter Geschwindigkeit unverändert. Dennoch konnten verschiedene Strategien bei den beiden experimentellen Bedingungen (Last und Geschwindigkeit) beobachtet werden, um die Arbeit am

Fußgelenk zu erhöhen; Mit Zusatzlast wurde eine höhere Nutzung elastischer Energie vorgezogen, wohingegen erhöhte Laufgeschwindigkeit in höherer Muskelaktivität resultierte. Die trainingsinduzierte Erhöhung der Achillessehnensteifigkeit beeinflusste ebenfalls die Muskel-Sehneninteraktion bei Ladungen und beim Laufen. Bei den Landungen war die abpuffernde Funktion der Sehne nicht geringer trotz erhöhter Sehnensteifigkeit und unveränderter Kraft. Die konkreten Mechanismen der Reduktion der Faszikelverlängerung von gastrocnemius und die größere Länge zu Beginn des Bodenkontakts bei der Landeaufgabe müssen in künftigen Untersuchungen ermittelt werden. Beim Laufen blieben die Verkürzungsmuster von gastrocnemius und soleus Faszikeln unverändert, obwohl der Rückschlag der Sehne verringert war. Das erhöhte architektonische Übersetzungsverhältnis des gastrocnemius während der Standphase konnte die asymmetrischen Änderungen von Sehnendehnung und –rückschlag erklären. Diese Interpretation legt zudem nahe, dass die elastische Energie nach dem Training auf andere Weise in der Sehne gespeichert wurde.

Im Rahmen der Versuchsbedingungen der vorliegenden Arbeit wurde das Muskelfaszikelverhalten bei Landungen und beim Laufen sowohl durch äußere Einschränkungen (d.h. Laufgeschwindigkeit und zusätzliche Last) als auch durch die Sehnensteifigkeit wenig beeinflusst, wobei soleus insgesamt weniger betroffen war als gastrocnemius. Die meisten Bedingungen waren stattdessen moduliert durch elastische Energiespeicherung oder durch Muskelaktivität. Wir vermuten, dass Energiespeicherung und Kontraktionsverhalten durch Änderungen der Steifigkeit der Aponeurose beeinflusst wurden und die vorliegenden Resultate deshalb nicht vollständig durch einfache Modelle, die Muskel und Sehne in Serie betrachten, erklärt werden können.

Amelie Werkhausen // The role of Achilles tendon properties in the mechanical function of the human triceps surae