Askild Vatnbakk Larsen

Effect of increased Achilles tendon stiffness on Gastrocnemius and Soleus fascicle behavior

Summary

Introduction: The contribution of tendinous structures and contractile elements to movement is often studied separately, and their roles considered independently. However, tendons and skeletal muscles are integrated, and in functional movements, the mechanical properties of both affect how they work together to produce movement (Lichtwark & Wilson, 2007). The Achilles tendon (AT) and the gastrocnemius (GM) and soleus (SOL) muscles are integrated, and the AT reduces muscular work by contributing to the majority of length change of the muscle-tendon unit (MTU), storing and recovering elastic energy. The mechanical properties of the AT affect GM and SOL mechanical work, and thus tuning tendon stiffness could affect the behavior of GM and SOL muscle fascicles.

Purpose: The purpose of this study was to examine if an exercise induced increase in AT stiffness affect GM and SOL fascicle behavior by reduction of shortening amplitude and contraction velocity. It was aimed to increase tendon stiffness while minimizing adaptations of the muscles.

Methods: 11 healthy individuals (age 25.7 ±4.2 years) formed the training group, and trained explosive isometric one-legged calf raises three times a week for 10 weeks. 10 controls (age 29 ±3.7 years) did not change their training habits during this period. Measurements of mechanical properties of the AT and resting muscle architecture of the GM was performed as well as visualization of the GM and SOL muscles during running using ultrasonography. Kinematics and kinetics of the hip and right leg was also measured during running.

Results: The TG increased AT tendon stiffness by ≈18% ($P = 0.0009$) and maximal plantarflexion torque by ≈15% ($P = 0.0013$), while the control group showed no change. Shortening amplitude and contraction velocity of GM and SOL fascicles showed no change in either group. The TG increased GM pennation angle change ($P = 0.0073$) and architectural gear ratio (AGR) ($P = 0.015$), while the control group showed no change.

Conclusion: Fascicle behavior did not change in the way expected. However, GM pennation angle change increased, thus increasing muscle shortening and AGR of the GM. As shortening of the muscle increased and a trend of increased dorsiflexion and lengthening of the MTU and EE was observed, EE strain may have increased, thus increasing utilization of elastic energy.

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Preface

After several years of having a personal interest in endurance running, both training athletes, and performing the sport myself, I am grateful to have been able to write my master thesis examining aspects of running that were new to me two years ago, and that I find very interesting. I would like to thank my main supervisor Olivier Seyennes and my co-supervisor Jens Bojsen-Møller for good help and guidance along the way. One of the traits that I have come to both enjoy and respect the most about you Olivier, is your ability to treat me as an equal, even though I sometimes feel like coming up short on knowledge and understanding of the subjects we discuss. Also very nice to have a supervisor that can come along for beach volleyball once in a while.

Special thanks goes out to Amelie Werkhausen, with whom I have been working closely with throughout this project. We've (at least I) have had my fair share of ups and downs during the past 1,5 years, and your good company and support along the way, both in the office, the lunch room, the beach volley court and via emails and phone calls have been invaluable. I am deeply impressed by your work moral, even though I still don't agree that more hours of work is always better. Thank you for your support, insight and a lot of good laughs!

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Askild Vatnbakk Larsen

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Introduction

The primary function of human skeletal muscle, from a mechanical point of view, is to convert chemical energy into mechanical energy to generate force and power (Frontera & Ochala, 2015). The Gastrocnemius and Soleus muscles are pennate muscles located posterior in the calf, contributing a substantial amount of force for propulsion in running (Farris & Sawicki, 2012).

The primary function of the human tendon is to transfer force produced by skeletal muscles to the human skeleton, to control and create movement of the joints (Witvrouw, Mahieu, Roosen, & McNair, 2007). The AT is the largest, thickest and strongest tendon in the human body (Benjamin, Kaiser, & Milz, 2008) (Doral et al., 2010), and is subject for substantial loads during running, with tensile forces having been measured to range from 3100-5330 N (Giddings, Beaupre, Whalen, & Carter, 2000; Scott & Winter, 1990).

The contribution of tendinous structures and contractile elements (CE) to movement is often studied separately, and their roles considered independently. However, tendons and skeletal muscles are integrated, and in functional movements, the mechanical properties of both affect how they work together to produce movement (Lichtwark & Wilson, 2007).

Running involves a stretch-shortening cycle of the muscle tendon unit (MTU) consisting of the Achilles tendon (AT) and the triceps surae (TS) muscles, located in the calf. During running, the AT and aponeurosis act as an energy conservator, storing elastic energy while lengthening and returning energy to the MTU during recoil (Cavagna, Saibene, & Margaria, 1964). Because elastic energy does not require production of ATP, the contribution of elastic energy from the tendon will contribute to increase running efficiency.

Like skeletal muscle, tendons also undergo adaptations to training stimuli. It has been shown that the stiffness of tendons increase with increased mechanical loading achieved through various forms of resistance training (Wiesinger, Kosters, Muller, & Seynnes, 2015). It has been suggested that variations in AT stiffness could affect the tendons capacity for storage and return of elastic energy to the triceps surae MTU, and thus affect running efficiency.

The mechanical and material properties of the AT have also been shown to modulate the time course of triceps surae muscle mechanical work (Albracht & Arampatzis, 2013; Lichtwark & Wilson, 2005a, 2006, 2007, 2008). It has been suggested that an increase in tendon stiffness will

reduce the extent of fascicle shortening and lowering contraction velocity. This would have implications for the amount of muscle volume being activated due to the nature of the force-velocity relationship of skeletal muscle (Hill, 1938). A reduction in contraction velocity would require activation of a smaller muscle volume to produce the same amount of force. A reduction in shortening of the fascicles will also reduce muscular work (Albracht & Arampatzis, 2013). Thus, tuning the stiffness of the AT could have the potential of reducing mechanical work of the attached muscles, and thus increase muscle efficiency.

Does the AT have an optimal stiffness to maximize work efficiency of the triceps surae during running? Lichtwark & Wilson (2007) report that optimal AT stiffness is equal to, or slightly higher than the average AT stiffness of a normal population.

Albracht & Arampatzis (2013) addressed the issue, examining how an exercise induced increase in AT stiffness affect gastrocnemius mechanical work. How increased AT stiffness affect Soleus mechanical work remains unexplored.

The intent of this master thesis is to examine how an increase in AT stiffness induced by training explosive isometric calf raises will affect the behavior of the medial gastrocnemius (GM) and soleus (SOL) muscle fascicles in running.

We hypothesise that:

1. An exercise induced increase in Achilles tendon stiffness will reduce the contraction velocity of the gastrocnemius and soleus muscle fascicles during stance in running
2. An exercise induced increase in Achilles tendon stiffness will reduce the shortening amplitude of gastrocnemius and soleus muscle fascicles during stance in running

Theory

Human skeletal muscle

The primary function of human skeletal muscle, from a mechanical point of view, is to convert chemical energy into mechanical energy to generate force. The main function of the force generated by the muscles is to maintain posture and produce movement.

At the whole-muscle level, the muscle is surrounded by connective tissue, the epimysium. Within the whole muscle, muscle fibers are then arranged in smaller bundles also surrounded by connective tissue (perimysium). At the level of a single muscle fiber, the fiber is surrounded by a cell membrane, the sarcolemma (Frontera & Ochala, 2015).
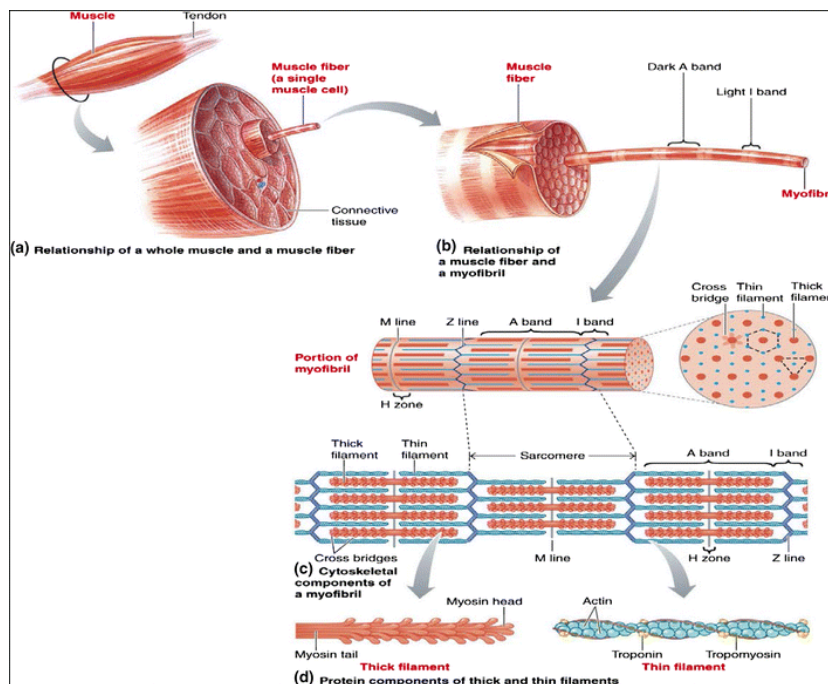

Figure 1

Illustration of the hierarchical architecture of skeletal muscles (Frontera & Ochala, 2015).

The bundles of muscle fibers surrounded by perimysium are known as muscle fascicles. The architecture of muscle fascicles vary between muscles, depending on the function and tasks the muscle is meant to perform. Architecture type is defined by the direction of the muscle fascicles

relative to the force-generating axis. Muscles with parallel architecture have muscle fascicles that are oriented along the force-generating axis. Muscles with this type of architecture consist of long muscle fascicles with a large number of sarcomeres in series and are suited for performing contractions at high speed, generating fast movement.

Pennate architecture is found in muscles where the orientation of the muscle fascicles is at an angle with the force-generating axis. The angle between the fibers and the force-generating axis is called the pennation angle. Pennate muscles exist as unipennate, bipennate or multipennate. Unipennate muscles have the muscle fibers oriented at an angle, all at the same side of a sheet of connective tissue called the aponeurosis. Bipennate muscles have fibers attached on both sides of an aponeurosis in the middle of the muscle. Multipennate muscles have fibers oriented at multiple angles relative to the force generating axis that are attached to several aponeuroses within the muscle. The architecture of pennate muscles allows for more muscle fibers within the volume of the muscle. This results in a larger physiological cross sectional area in relation to anatomical cross sectional area, increasing maximal force production per muscle volume. Another consequence of pennate architecture is a shorter fascicle length than in muscles with parallel architecture. A short fascicle length is beneficial for economical force production, but at the expense of the ability to produce force at high contraction velocities. Thus, pennate muscles with a short fascicle length are economical and have a high force-producing capacity per volume, but have a limited capacity to produce high force at fast contraction velocities (Marco Narici, 1999)(Zatsiorsky & Prilutsky, 2012) (McGinnis, 2013).
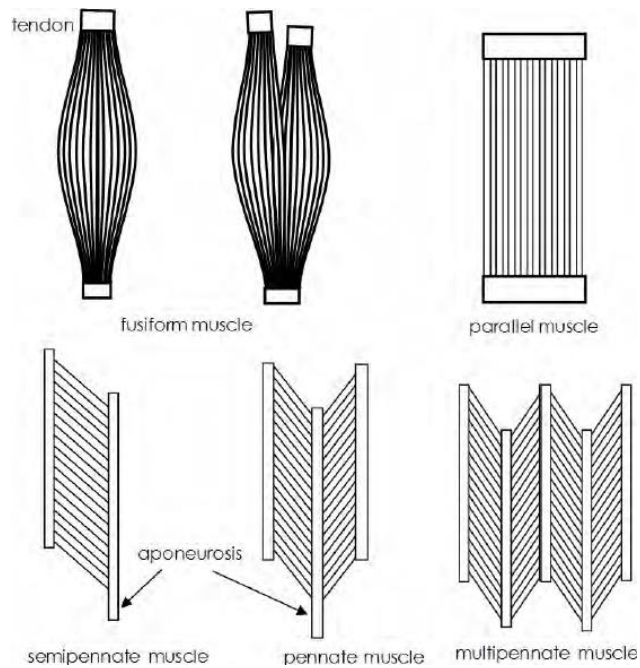
Figure 2

Illustration of muscles with fusiform, parallel and pennate architecture (Nishihara et al., 2012)

The medial gastrocnemius muscle (GM) further examined in this master thesis, is a unipennate muscle. The GM is attached to the medial condyle of the femur and runs down on the medial side of the calf where it forms a common tendon with the soleus, the AT. The soleus (SOL) muscle also examined in this thesis is located deeper in the calf, beneath the GM. The SOL muscle is a multipennate muscle that attaches just below the knee and runs down to form the AT, common with the GM muscle.
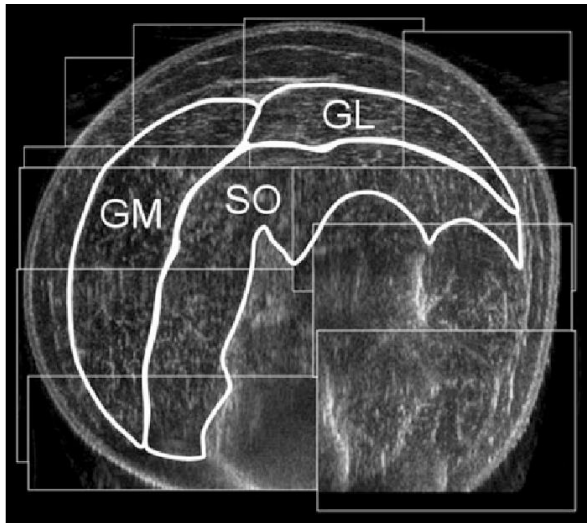
Figure 3

Ultrasonographic image of the triceps surae muscles, including the gastrocnemius medialis (GM), gastrocnemius lateralis (GL) and the soleus (SO) muscles. (Foure, Nordez, McNair, & Cornu, 2011)

The GM muscle is a biarticulate muscle, performing flexion of the knee and plantar flexion of the ankle. The SOL muscle's function is plantar flexion of the ankle. Studies examining the distribution of net positive power produced over the hip, knee and ankle during running, find that the muscles creating power over the ankle joint is responsible for >40% of total net positive power production (Farris & Sawicki, 2012), showing that the plantar flexors are a major contributor of propulsive forces during running. The complexity of demands that the GM and SOL muscles needs to meet during running make their properties of great importance for running efficiency.

The GM and SOL muscles is faced with a tricky challenge. During running, they need to produce a substantial amount of force, fast, because of the limited amount of time the foot spends on the ground. At the same time they are desired to be as economical as possible, requiring short muscle fascicles (Lichtwark & Wilson, 2008).

Muscle gearing

Though pennate muscles like the GM and SOL does not have a large number of sarcomeres in series suited for fast contraction speeds, they have a means for being able to perform fast contractions of the muscle. During contraction of a pennate muscle, the pennation angle changes to a more obtuse angle relative to the force generating axis (FGA). Muscle fiber rotation relative to the FGA contributes to the shortening of the whole muscle belly, reducing the demand for high contraction speeds within the fascicles. The ratio between the contraction velocity of the whole muscle belly and the velocity of the fascicles is known as muscle architectural gear ratio (AGR). AGR varies between muscles with different fiber architectures. Muscles with parallel architecture have close to equal fascicle and whole- muscle contraction velocity during contraction. Pennate muscles show a different relationship. Because of the angle of the fibers relative to the FGA, the whole muscle belly will shorten less per unit of shortening of the fascicles. Fiber rotation in pennate muscles will shift this relationship towards more muscle shortening per fascicle shortening. Increased fiber rotation is synonymous with an increased AGR. "The magnitude of fiber rotation, and therefore gear ratio, depends on how the muscle changes shape in the dimensions orthogonal to the muscle's line of action" (Azizi, Brainerd, & Roberts, 2008) (Dick & Wakeling, 2017).

It has been suggested that AGR can vary within the same muscle. Depending on the type of contraction being performed, pennate muscles show different changes in muscle-shape during contraction, resulting in a different extent of change in pennation angle (fiber rotation). It has been found that dynamic muscle-shape changes cause increased fiber rotation at low forces and less fiber rotation at high forces. The mechanism behind variations in muscle-shape changes dependent on contraction force is unclear. It has been suggested that it is a product of intramuscular forces and their pressure on connective tissues within the muscle. This means that for high-load, slow contractions, the pennation angle will change less. This would be beneficial as the fascicles will be able to handle the relatively low demands for contraction speed, and the direction of the force produced by the contractile elements will be directed at a smaller angle relative to the FGA, transferring a larger percentage of the force in the FGA direction. For low-load fast contraction, dynamic muscle-shape changes promote pennation angle changes to a greater extent. This will reduce the maximal capacity to generate force along the FGA because of a more obtuse pennation angle throughout contraction. On the other hand, it allows the muscle

fascicles to operate at lower contraction speeds because of the contribution of fiber rotation to the shortening of the whole muscle belly. A higher AGR leaves the pennate muscle able to produce a greater amount of force at higher contraction velocities (Azizi et al., 2008).
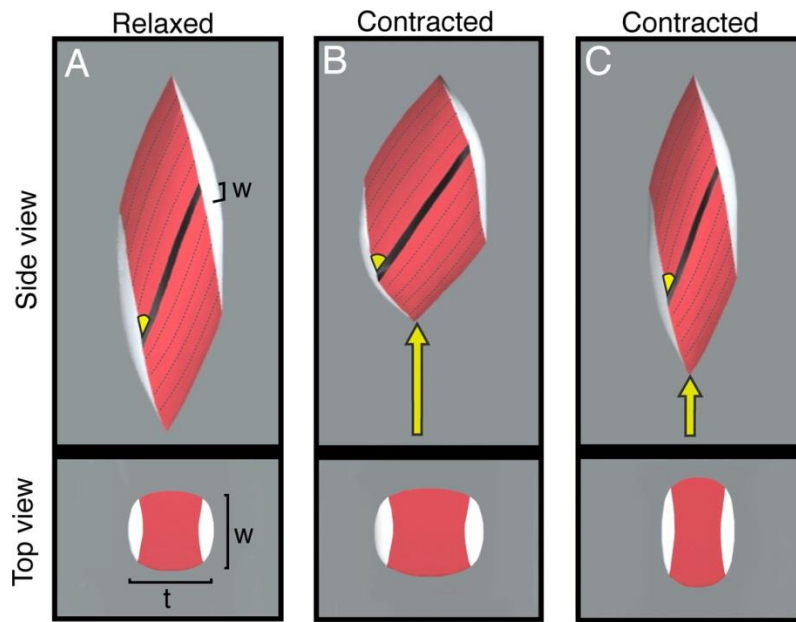
Figure 4

Illustration of how a pennate muscle display varying dynamic muscle-shape changes during different types of contractions, resulting in more (B) or less (C) fiber rotation (Azizi et al., 2008)

Tendons

Tendons are fibrous tissue attached to a muscle on one side and the skeleton on the other side, transferring force produced by the muscle to the skeleton to produce movement and/or stability over joints (Thorpe & Screen, 2016). Tendons consist of extracellular substance, cells and water. 55-70% of the content in tendinous tissue is water. Extracellular substance consists of collagen, elastic fibers and ground substance. Ground substance consists of glycoproteins, plasma proteins and proteoglycans. Proteoglycans are responsible for binding water to the extracellular substance, giving the tendon its viscous properties. Type 1 collagen make up the majority of collagen in the tendon (≈60%).

In much the same way as muscles, the tendon consists of bundles of fibers arranged in a hierarchical fashion. The largest bundles of fibers are called tertiary bundles. Tertiary bundles of

fibers consist of several secondary bundles that in turn consist of primary bundles of fibers. The primary bundles consist of collagen fibers. Collagen fibers are made up of collagen fibrils that in turn are made up of triple-threaded collagen molecules, containing three collagen polypeptide chains. The majority of the tendons dry mass is collagen (60-85%) (Kirkendall & Garrett, 1997; Kjaer, 2004).
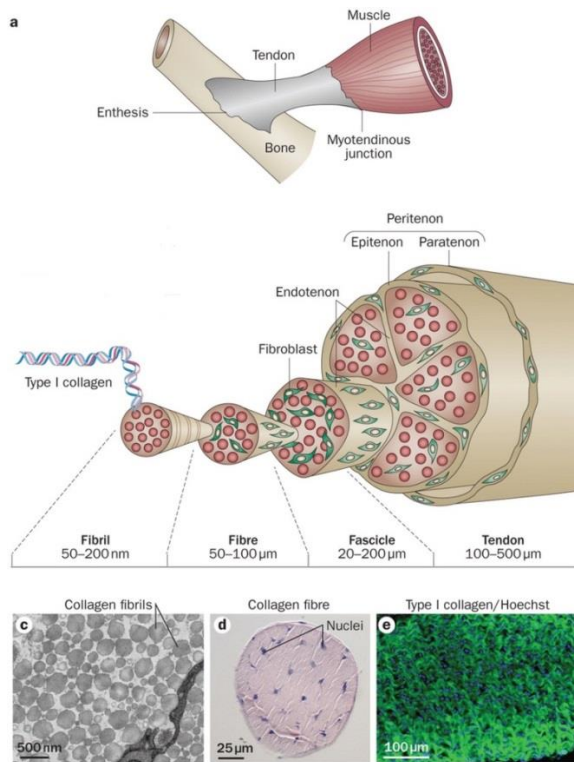
Figure 5

Illustration of a tendons hierarchical architecture and attachment to muscle and bone (a) as well as microscopic view if collagen fibrils (c), collagen fiber (d) and type 1 collagen (e). (Nourissat, Berenbaum, & Duprez, 2015)

Elastic fibers make up 1-2% of the dry mass of the tendon. These fibers have been suggested to have the function of resetting the collagen fibers to their original structure after muscle contractions. The structure of the collagen fibrils is three dimensional, being oriented in both the longitudinal, transversal and horizontal plane relative to the muscle force. Because of this three dimensional structure, tendons are capable of absorbing both longitudinal, horizontal, transversal and rotational forces applied during different types of locomotion (Kannus, 2000)

Material and mechanical properties of tendons

In addition to functioning as a force transmitter from the muscle to the skeleton, tendons have material and mechanical properties that optimize human movement. An elastic property is defined by an object being able to return to its original form after undergoing deformation. A viscous property, on the other hand, is defined as an object *not* returning to its original form after deformation. Tendons exhibit both of these properties, making tendons a viscoelastic material. The elastic properties of tendons allow them to stretch, and store elastic energy in the process. The elastic energy is released during recoil, as the tendon returns to its original length.

 The viscoelastic properties of tendons results in the relation between forces applied to the tendon, and tendon deformation not being linear throughout an elongation from original length to, ultimately, failure. At the start of deformation, less force is required to stretch the tendon a given length. This is known as the toe-region (≈2% of elongation). Less force is required to stretch the tendon in the toe-region because the fibers are being stretched from a wave-like form. When the fibers are stretched into a straight form, the tendon will enter the linear region, where the relation between force applied and deformation is consistent (≈2-4% of elongation). As stretch of the tendon continues beyond 4% of elongation, the tendon enters the plastic region, where the stretch is too much for the tendon to go back to its original length. In this region, micro ruptures of fibres can occur. If the tendon reaches an elongation beyond 8%, macro ruptures can occur. Elongation beyond 8% puts the tendon at risk of a complete rupture (Doral et al., 2010; Maganaris, Narici, & Maffulli, 2008).

Measurements

The length (cm), elongation (cm), and cross-sectional area (mm^2) of the AT can be measured by ultrasound. These measurements have been used in numerous previous studies to calculate the stiffness, stress and strain of the AT (Albracht & Arampatzis, 2013; Lichtwark & Wilson, 2005b, 2006).

- Tendon stiffness = ΔForce / ΔElongation
- Stress = Force / Area
- Strain = Elongation / Original Length

Assuming identical material properties and moment arm of the AT, a tendon with a lower CSA will be more compliant than one with a higher CSA. Applying the same force to these identical tendons would result in more elongation of the tendon with a lower CSA.

The material properties of a tendon vary between individuals, affecting tendon stiffness. The stress (force/area) - strain (elongation/initial length) curve is called Young's Modulus and reflects the material properties of the tendon, making it possible to compare tendons of varying length and CSA. The slope of the curve will describe the material stiffness, a steeper slope reflecting a stiffer material of the tendon. Tendon stiffness is usually measured in the linear region and is affected by the tendons length, CSA and material properties.
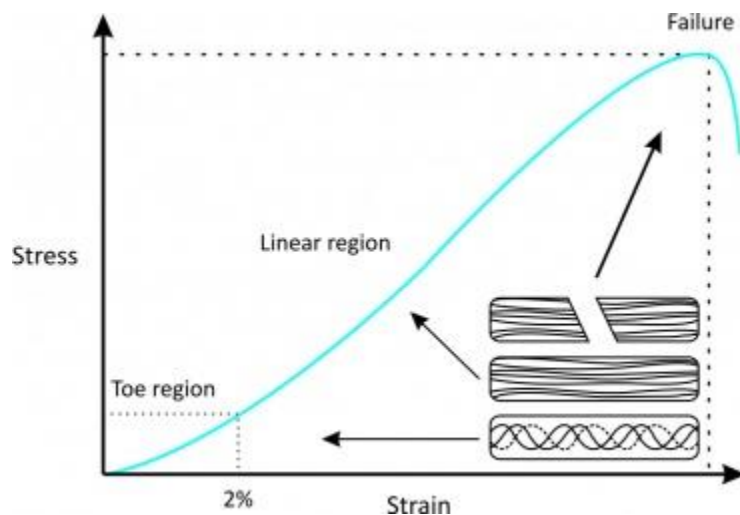

Figure 6

Stress – strain relationship (Young's Modulus) of tendons (Robi et.al, 2013)

Measurements of the tendons material and mechanical properties

To calculate the material and mechanical properties of tendons, the CSA, length and deformation needs to be measured. The force causing the deformation of the tendon also needs to be measured and synchronized with the deformation.

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In vitro

These parameters have historically typically been measured in vitro. In vitro measurements involve taking biopsies of tendons. The biopsies are stretched with a known force, and fascicles of the tendons are examined with a stereomicroscope, or by atomic force microscopy. Tendons have been shown to not have a uniform stiffness along their entire length, and in vitro measurements have the advantage over in vivo measurements of being able to measure the tendons stiffness at different locations along the tendons length. In vitro measurements also have the advantage of more precisely measuring the force acting on the tendon as the tendon is stretched with a known force. Biopsies have to be treated and stored in various ways before performing measurements. This could change the mechanical properties of the biopsy as compared to its actual properties in vivo, highlighting a disadvantage concerning in vitro measurements.

In vivo

These parameters have since 1995 been examined in vivo using ultrasound. In vivo measurements are most often done with B-mode ultrasonography, with subjects performing isometric ramped contractions where change in the deformation of the tendon (using ultrasound) and isometric torque are synchronized (Arya & Kulig, 2010; Reeves, Maganaris, & Narici, 2003). Tendon force is calculated during ramped isometric contractions, and is a product of the externally measured joint moment, the internal moment arm and taking into account the co-activation of antagonist muscles. In vivo measurements of tendon mechanical properties have shown a greater variance than cadaveric studies. The reason for this is suggested to be a large variance in methodological approaches. Depending on the methodological approach of choice, there are potential sources of error. Because ultrasound transducers have a limited field of view, a common approach of measuring deformation of the tendon is to visualize the displacement of only the myotendinous junction, assuming that the displacement of the distal tendon insertion is negligible, introducing a potential source of error. Most studies use two dimensional ultrasound scanning that captures tendon deformation only in the longitudinal direction. However, tendons are three dimensional structures that undergo deformation in all three dimensions. Not taking into account the three dimensional structure and deformation of tendons leads to an

underestimation of tendon length and an overestimation of length change of the tendon. In contrast to in vitro measurements, where the tendinous structure is stretched with a known force, in vivo measurements rely on estimations of forces acting on the tendon. To estimate the force exerted on the tendon, the internal moment arm is also estimated. This is done in various ways, all having limitations and potential sources of error (Seynnes et al., 2015).

However, in vivo measurements of tendon mechanical properties has been shown to be reliable when taking the proper precautions are taken. The introduction of a non-invasive in vivo measurement method makes it possible to examine tendon mechanical properties during real-life physiological conditions, a big advantage for gaining understanding of tendon function in a large spectrum of real-life locomotion (Seynnes et al., 2015).

Adaptation to mechanical stress

Tendons, being a passive structure, have historically been believed not to undergo adaptation to mechanical stress (MS). Different levels and type of activity has been shown to result in large variations in cross sectional area and traits of skeletal muscle. It would be logical to assume that tendons attached to the muscle would need to undergo adaptation reflecting those of the muscle to meet the demands of the stimuli being inflicted. Specific traits of the muscle are optimal for different athletic endeavors and it would be natural to assume the same applies for tendinous tissue. Tendinous tissue has been shown to have a significantly lower metabolic rate than skeletal muscle (Boushel et al., 2000), and as a consequence tendons undergo adaptation to MS significantly slower than skeletal muscle. Insufficient duration of intervention studies could be a reason for the early assumptions that tendinous structures remain unaffected by training stimuli.

Even though tendons have a low metabolic rate and limited vascularity, they undergo material, mechanical and morphological changes when exposed to increased MS. In studies in recent years using ultrasonography, tendons have been shown to respond to training, undergoing changes in their material and mechanical properties (Arampatzis, Karamanidis, & Albracht, 2007; K. Kubo, Ikebukuro, Yata, Tsunoda, & Kanehisa, 2010b).

"Mechanical load induced as cyclic strain, imposed externally to fibrous connective tissues such as tendons, may induce several signals at the extracellular matrix. This happens through mechanotransduction pathways affecting the anabolic as well as the catabolic responses" (Arampatzis et al., 2007).

Tendons respond to increased loading by increasing collagen synthesis (Langberg, Rosendal, & Kjaer, 2001; Langberg, Skovgaard, Petersen, Bulow, & Kjaer, 1999). An increase in enzymes in the extracellular matrix controlling collagen turnover, as well as an increased expression of growth factors has also been observed. Collagen molecules that are synthesized in this process are then believed to be added to the existing fibrillar structure. This way, the tendon is able to change material and mechanical properties to meet the demands of the amount and type of loading they frequently are exposed to.

Increased tendon stiffness could be the result of both changes in the tendons material properties and a larger tendon cross sectional area (CSA). A meta-analysis conducted by Wiesinger et al (2015), including thirty-five peer reviewed articles found evidence that tendons respond to increased MU with increased stiffness as early as after 6-8 weeks. Short term adaptations of increased stiffness were often accompanied by an increase in young's modulus, without any increase in the tendon CSA. This indicates that increased tendon stiffness as a result of a short-term training intervention (weeks) of increased mechanical loading results primarily from changes in material properties. Increasing the CSA of a tendon seems to be a slower process than changing material properties. Some training studies have reported tendon hypertrophy, but not on a large scale ($\approx 5\%$). To achieve significant hypertrophy of the tendon requires years of systematic loading. This is indicated by athletes performing sports with significant loading of tendons over a number of years showing as much as a 20% larger CSA than control subjects (Wiesinger et al., 2015).

Tendon morphology and function

Tendons exist in various sizes and shapes in the human body. Looking at the morphology of different MTU's in the human body, it becomes apparent that they are highly specialized for the tasks they are meant to perform. In general, tendons located more proximally in the lower limbs

make up a relatively small part of the MTU's total length. Tendons in these types of MTU's have little capacity for deformation, and thus little capacity of elastic energy storage. Their specialty and primary function is to effectively transfer force produced by the attached muscle to the skeleton to produce movement (Farris & Sawicki, 2012).

Tendons located more distally in the lower limbs show different characteristics. In general they are more compliant, longer, and make up a larger part of the MTU's total length. MTU's located distally in the lower extremities need to be versatile and meet the demands of a broad range of locomotion. Having a long, compliant tendon that makes up a large part of the MTU contributes to versatility and ability to meet these demands (Doral et al., 2010).

The viscoelastic properties of tendons are essential to perform functional tasks. These tasks include absorption of energy to protect the muscles from doing too strenuous work during landing tasks (Werkhausen et al., 2017). Tendons also work as power amplifiers when jumping, utilizing force produced from the attached muscles to store elastic energy in the lengthening phase, and returning energy to the MTU when the length of the muscle is too short for optimal force production (Sawicki, Sheppard, & Roberts, 2015). Tendon elasticity has also long been suggested to reduce the energy cost of locomotion involving a stretch-shortening cycle (Cavagna, Heglund, & Taylor, 1977).
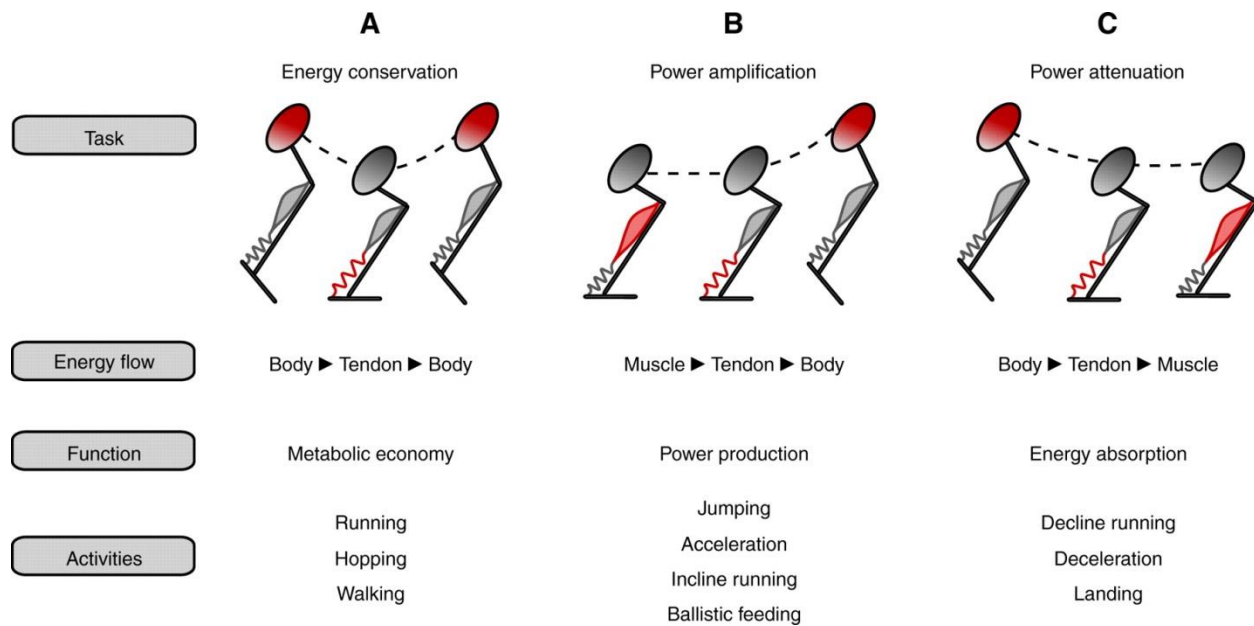
Figure 7

Illustration of the TS MTU performing energy conservation (A), Power amplification (B) and Power attenuation (C) (Roberts & Azizi, 2011)

The MTU consisting of the Achilles tendon (AT) and the triceps surae (TS) muscles is an example of this. The AT further examined in this master thesis origins from the soleus and gastrocnemius muscle posterior in the calf, and attaches at the calcaneus. The primary function of the AT is to transfer force from the triceps surae muscles to the calcaneus, performing plantar flexion of the ankle. Due to the viscoelastic properties of the AT and aponeurosis, it is multifunctional, and provides several critical functions during human locomotion (Egger & Berkowitz, 2017). The AT is the largest, thickest and strongest tendon in the human body (Benjamin et al., 2008) (Doral et al., 2010), and is subject for substantial loads during running, with tensile forces having been measured to range from 3100-5330 N (Giddings et al., 2000; Scott & Winter, 1990). The length of the AT ranges from 110-260mmand its shape changes in the longitudinal direction. At the proximal insertion, the AT is broad and flat, and it becomes thinner towards its mid-portion, where it has more of a cylindrical shape. Distally, it becomes broader again until its insertion at the calcaneus (Doral et al., 2010). The AT makes up a large part of the total length of the TS MTU is responsible for the majority of the MTU's length change during activities that involve a stretch shortening cycle.

During running, the AT acts as an energy conservator, storing elastic energy while lengthening and returning the stored energy to the MTU when shortening. Because this energy does not require production of ATP, the energy conservation performed by the AT will contribute to an increased running efficiency (Wilson & Lichtwark, 2011).
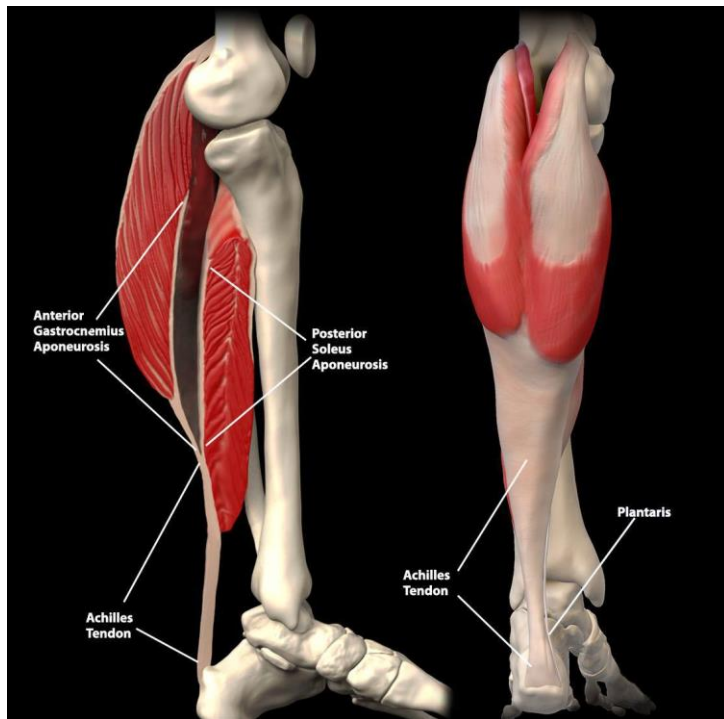
Figure 8

Illustration of the Triceps Surae MTU (Stadnick, M.E, 2016)

Muscle-Tendon interaction and running

Studies examining the behavior of the fascicles of the gastrocnemius muscle (GM) during running find that the GM fascicles are continuously shortening during the stance phase, while the tendon and the whole muscle-tendon unit lengthened during the first half of stance (Ishikawa, Pakaslahti, & Komi, 2007; Lichtwark, Bougoulias, & Wilson, 2007). This implies that work done by the muscles as well as potential and kinetic energy of the human body are stored within the tendon during the first phase of stance. In theory, a more compliant AT would store and return more elastic strain energy, which is beneficial for running efficiency. Consequently, it could be argued that tendon stiffness higher than what is required for a certain running speed could be detrimental for running efficiency (Albracht & Arampatzis, 2013; Lichtwark & Wilson,

2008). Several studies have examined the relation between tendon stiffness and running economy and performance. The results are conflicting, with some studies showing a stiffer tendon to be beneficial for performance or economy (Albracht & Arampatzis, 2013; Fletcher, Esau, & MacIntosh, 2010), while others show a more compliant tendon to be more beneficial (Keitaro Kubo, Miyazaki, Shimoju, & Tsunoda, 2015). Of these studies, Albracht & Arampatzis 2013 is the only one considering behavior of muscle fascicles (GM). A fundamental difference between these studies is that some are examining the relationship between tendon stiffness and performance over a certain distance (Kubo et al 2015), while the remaining studies examine the relationship between tendon stiffness and running economy at predetermined running speeds. Only Albracht &Arampatzis (2013) have performed a training intervention with the intent of increasing tendon stiffness, while the other studies are cross-sectional, measuring tendon stiffness and running economy in one session. Treating the tendons abilities to utilize elastic energy alone do not work very well when the context is a real-life movement like running. The AT acts as a part of the MTU, and optimal tendon stiffness for maximizing running efficiency in a real-life context does not only concern the AT's ability to store and return elastic energy alone, but also how the properties of the AT affect the mechanical work of the attached muscles.

Assuming the same forces are acting on the tendon and morphology of the muscle-tendon unit being the same, a stiffer tendon and aponeurosis would experience less strain, leading to a decrease in shortening of the fascicles and therefore a decrease in work performed by the muscles. Consequently, fascicle shortening velocity would also be reduced. The work performed by the muscles would then be more efficient due to the nature of the relationship between force production and contraction speed of the muscles (Hill 1938).
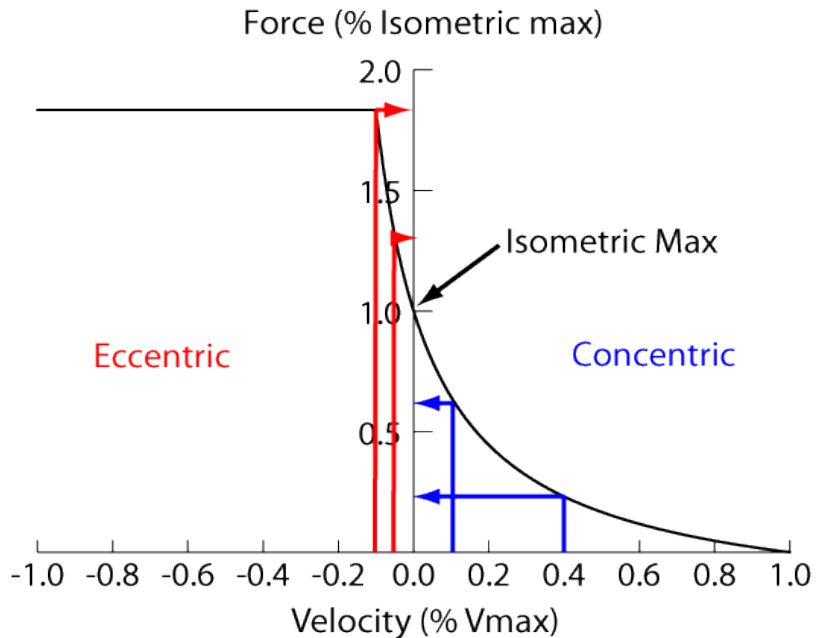
Figure 9

Illustration of the Force – velocity relationship of skeletal muscle ("Muscle force – velocity relationship", 2014)

As contraction speed decreases, a smaller amount of muscle fibers recruited would be required to produce the same amount of force. Assuming the hysteresis of the tendon stays the same, the energy loss would be less in a stiffer tendon and aponeurosis. It could therefore be argued that a stiffer tendon and aponeurosis would increase running efficiency by reducing muscular work and reducing energy loss (Albracht & Arampatzis, 2013).

In general, less sarcomeres in series i.e. a shorter muscle fascicle is more optimal for minimizing activation cost (Lichtwark & Wilson, 2008). At the same time, the muscle fascicle needs to be long enough to produce the enough force at the shortening velocities required by the movement at hand. A trade-off between these two attributes exists in muscles that are required to perform fast contractions to produce movement and at the same time are desired to produce the movement as energy efficient as possible. This situation applies for the muscles of the triceps surae during endurance running. Even at submaximal speeds, the foot has ground contact for a limited time, requiring the muscle to produce force at high shortening velocities. At the same time, the movement is desired to be as energy efficient as possible, requiring the muscle to minimize activation cost (Lichtwark & Wilson, 2008).

To provide a better understanding of the integration of muscles and tendon structures, computational modelling has been used to gain understanding of how the two structures would affect each other if their mechanical properties are altered (Lichtwark & Wilson 2008). As opposed to traditional cross-sectional or intervention studies, using modelling allows for immediate manipulation of the mechanical properties of both tendinous structures and the attached muscles. As examined by Lichtwark & Wilson 2008, tendon stiffness alter the muscles mechanical work, but different degrees of tendon stiffness could have different consequences depending on the fascicle length of the muscle attached to the tendon. It is suggested that the optimal stiffness of the tendon for efficiency is dependent on the fascicle length of the attached muscle and vice versa. A longer muscle fascicle would in general require a stiffer tendon for optimal efficiency, and a shorter muscle fascicle would require a more compliant tendon. The optimal relationship is also affected by the speed of running. Faster running would in general require a stiffer tendon and a longer fascicle, while running at slower speeds would require a more compliant tendon and a shorter muscle fascicle to maximize muscle efficiency (figure 10) (Lichtwark & Wilson, 2008).
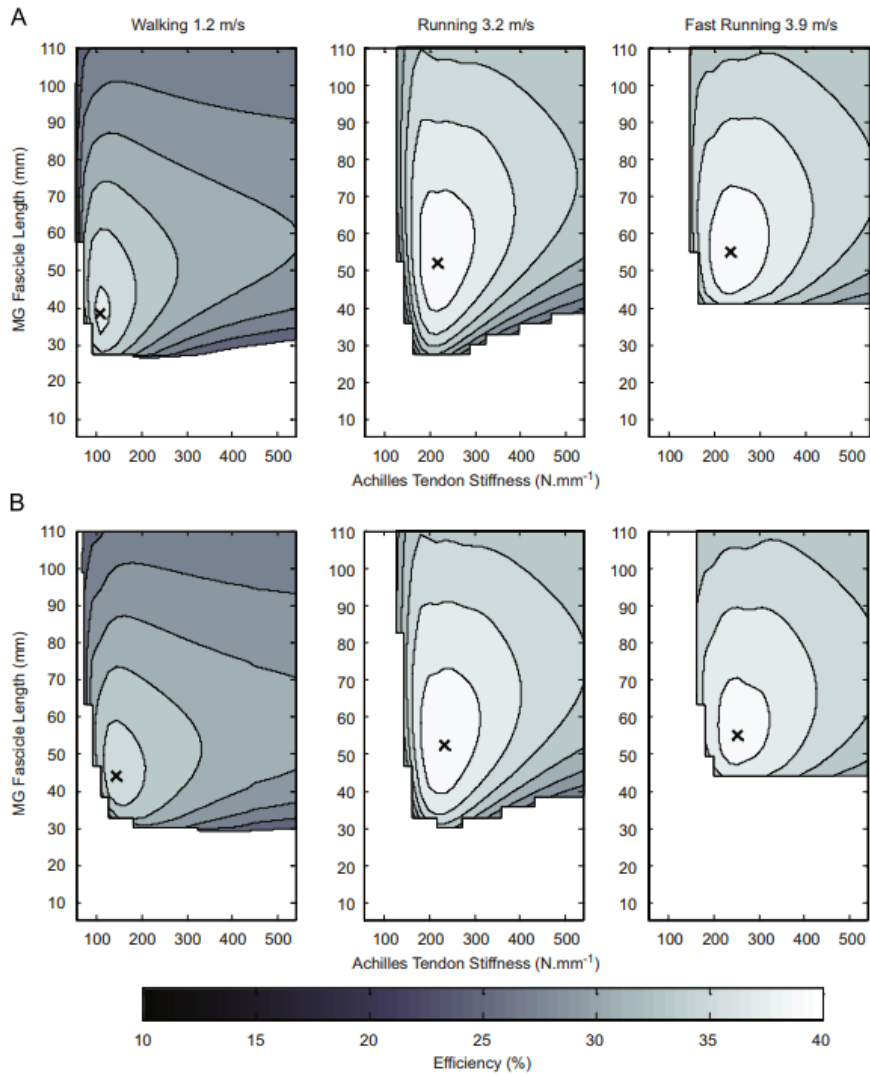
Figure 10

Illustration of the relationship between tendon stiffness, medial gastrocnemius (MG) fascicle length and muscle efficiency at different speeds (Lichtwark & Wilson, 2008)

Methods

Twenty-one healthy individuals participated in the study after giving informed consent to the experimental procedure, complying with the rules of the Helsinki-declaration and the ethical committee of the Norwegian School of Sport Sciences. Subjects recruited to the training group (TG) were required to have a training history of no strength training for the calves. No restrictions were put on the training history of the subjects participating in the control group (CG). Eleven healthy participants (mean, ±SD), age 25,7 ±4,2 years, height 174,2 ±9,7 cm, and body mass 70 ±9,8 kg were recruited to the TG. The remaining ten participants formed the CG, age 29 ±3,7 years, height 176,7 ±9,8 cm, and body mass 72,3 ±9,4 kg. The TG performed explosive isometric calf raises for a period of 10 weeks, while the CG did not change their training habits during this period. The subjects were tested before and after the training intervention in one testing session. The testing session included the following measurements, which are described in detail later: 1: Mechanical properties of the MTU. 2: Resting muscle architecture of the GM (fascicle length, pennation angle and muscle thickness) and the AT (CSA and length) with the ankle joint in the anatomical neutral position (0°)). 3: Kinetics and kinematics of the hip and the right lower extremity during running as well as ultrasound imaging of the Gastrocnemius (GM) and the Soleus (SOL) fascicles and aponeurosis muscle during running. The CG performed the same tests as the TG.

Exercise protocol

The participants in the TG trained single-legged explosive isometric calf raises 3 times per week for a period of 10 weeks. The calf raises were performed in 4 sets of 10 repetitions each (1s loading, 5s relaxation). Prior to contraction, the ankle was slightly dorsiflexed, the knee joint fully extended, and the hip joint at 0°. The participants performed each plantar flexion to 80% of their maximal voluntary contraction (MVC). MVC's were performed every third training session, and the load of the repetitions was increased as the strength of the subjects progressed during the training period. Repetitive isometric ankle plantarflexion contractions were chosen to induce cyclic strain on the TS tendon and aponeurosis (Arampatzis et al., 2007) and increase Achilles Tendon stiffness. Explosive contractions with a limited duration was chosen to minimize muscle hypertrophy by reducing time under tension as compared to classical dynamic

training with added load. The small operating range around anatomical position during contractions was chosen to avoid changes in sarcomeres in series pre to post intervention.
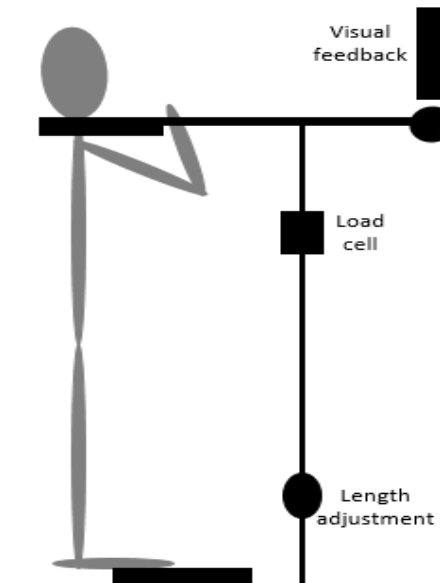
Figure 11

Illustration of the apparatus used for the exercise intervention (Werkhausen, 2018)

Mechanical properties of the Achilles tendon

First, the subjects performed a 5 min warm up, running barefoot on a treadmill at personally preferred speed before further measurements. Resting muscle architecture was imaged by ultrasound (LS 128 Telemed, Vilnius, Lithuania). Images were taken with the subjects lying prone with the hip, knee and ankle joints in anatomical neutral position. Thickness, fascicle length and pennation angle of the gastrocnemius medialis (GM) and soleus (SOL) muscles were measured offline with software for image analysis (ImageJ, National Institutes of Health, Bethesda, USA). The shortest distance between the two aponeurosis at 25, 50 and 75% of the width of the ultrasound image was averaged and used to represent muscle thickness. A straight line aligned with visible muscle fascicles between aponeurosis defined fascicle length. In cases where fascicles exceeded the field of view, linear extrapolation of the fascicle to the aponeurosis was performed. The angle between the measured fascicle and the orientation of the deep aponeurosis defined the pennation angle.

The contractile strength of the plantar flexors was measured by performing 2-3 MVC's with 1-2 min rest between contractions. The subjects lay prone with the ankle firmly attached to a dynamometer (IsoMed 2000 D&R Ferstl GmbH, Hemau, Germany) in the anatomical neutral position (0°). The rotation axis of the dynamometer and ankle joint were aligned before the test. A solid foam pad was put under the subject's knee to ensure the knee joint was fully extended during contractions. As a specific warm up, the subjects performed at least 5 submaximal contractions of the plantar flexors.

To determine the Achilles tendon stiffness, a series of ramp-contractions was performed. Ultrasound scans (LS128, Telemed, Vilnius Litauen, framerate 80 Hz) of the gastrocnemius myotendinous junction, plantarflexion torque (600 Hz) and marker trajectories (120 Hz) (Qualisys, Gothenburg, Sweden) were recorded simultaneously during contractions to estimate tendon stiffness. Measurements were synchronized using a trigger signal from the ultrasound system. To eliminate movement of the ultrasound transducer, it was fixed at the myotendinous junction using self-adhesive tape and elastic bands. To ensure consistent scanning when the muscle was bulging, a gel pad was placed between the transducer and the skin.

Lying prone in the same position, the subjects were asked to gradually increase the torque up to 90% of MVC. The subjects had visual aid of the slope at a loading rate of 100 N·m s^{-1} up to the point of 90% of MVC on a screen as they performed the contractions. Before trials were recorded, the subjects performed ramp contractions until they were sufficiently familiarized with the task. Two Qualisys super-spherical passive markers were attached to the subject's calcaneus and medial malleolus. Three additional markers were attached to the ultrasound probe to know the location of the probe in the laboratory coordinate system and to detect any unwanted movement of the probe. The movement of the markers was registered by 4 cameras mounted at different heights and angles around the dynamometer to ensure registration of all markers throughout the movement. The area where subjects performed the test was calibrated using a calibration wand with a width of 499.7 mm before each test.

Running measurements

Subjects performed their running barefoot on a treadmill (Force-Link, Motek, Netherlands) without incline at the same speed they preferred during warm-up.

In total, eighteen Qualisys super-spherical passive markers were attached to the subject's right leg and hips. To define the center of the hip joint, markers were placed at the left and right (anterior and posterior) iliac spine. The center of the knee joint was defined by markers on the medial and lateral condyle of the knee joint, while the centre of the ankle joint was defined by markers attached to the medial and lateral malleolus To define movement in the foot segment, markers were attached to calcaneus and the first, second and fifth head of the metatarsals. The thigh and shank segments were traced by marker-clusters consisting of four markers each attached laterally to the thigh and shank.

The movement of the markers was registered by 15 cameras (Qualisys, Gothenburg, Sweden) mounted at different heights and angles around the treadmill to ensure registration of all markers throughout the movement. The area around the treadmill was calibrated using a calibration wand with a width of 749,2 mm (Qualisys, Gothenburg, Sweden) before the start of running trials. The system was judged properly calibrated with the criteria that the length of the calibration wand, which was measured by the capture system, was within 0.10 mm of the true wand length.

The laboratory-coordinate system used for the study was set up as follows: X-axis in the medial-lateral direction and positive pointing to the right. Y-axis in the direction of gait progression and positive pointing forward. Z -axis in the vertical direction and positive pointing upward.

Ground reaction forces were measured with a cut-off frequency of 25 N using a force platform integrated in the treadmill used for running trials (Force-Link, Motek, Netherlands).

Ultrasound scans (LS128, Telemed, Vilnius Litauen, framerate 80 Hz) of the medial gastrocnemius and soleus muscle were recorded during running trials of at least 10 step cycles to visualize fascicles and aponeuroses. To minimize movement of the ultrasound transducer, it was placed in a custom-made holder and fixed at the medial gastrocnemius muscle belly using self-adhesive tape and elastic bands. A trigger signal from the ultrasound system synchronized all measurements.

Figure 12

Running in the lab on the force-plate instrumented treadmill with the ultrasound-transducer and kinematic markers attached

Data analysis

Maximal torque and tendon mechanical properties

Filtering of marker trajectories was performed with a second order bidirectional low-pass Butterworth filter (cut-off frequency 15 Hz). Tracking of the GM myotendinous junction (MTJ) position was done offline semi-automatically by tracking the closest fascicle insertion (Tracker 4.95, physlets.org/tracker/). A second order bidirectional low-pass Butterworth filter with a cut-off frequency of 6 Hz was used to filter ultrasound data. The position of the ultrasound image relative to the kinematic markers on the cast used to hold the ultrasound transducer was established by prior calibration, enabling calculation of the position of the MTJ in the laboratory coordinates system. The distance between the myotendinous junction and the calcaneus marker was used to calculate AT length during the contraction (Gerus et al., 2011). The same filtering as applied to the kinematic data was applied to plantarflexion torque data. Estimation of AT force was calculated dividing the plantar flexion torque with the internal moment arm of the AT. The internal moment arm was measured with a tape measure externally, using the mean perpendicular distance from the tendon to the midpoint between the medial and lateral malleolus.

To represent the proportion of total ankle moment attributable to the triceps surae, 91% of the calculated tendon force was used (Dick, Arnold, & Wakeling, 2016). The torque was corrected to account for ankle joint rotation and gravitational forces (Karamanidis et al., 2005) using kinematics. The tendon force-elongation plots of three out of five trials were averaged and fitted with a third order polynomial, with the highest and lowest stiffness excluded. For every subject, tendon stiffness was calculated as the slope of the force-elongation curve between 50% and 80% of the maximum force level. Maximum tendon strain was measured at the maximum common force of pre- and post-intervention test for every subject.

Running kinematics and kinetics, muscle mechanics

Analysis of kinematic and kinetic data was done using a standard Newton-Euler inverse dynamics procedure (Visual 3D, C-Motion Inc., Germantown, MD, USA). Angles, moments and power of the hip, ankle and knee joints were expressed in the coordinate system of the respective proximal segment. The product of the joint moment and the joint angular velocity was used to calculate power of the ankle, knee and hip joints. Data was filtered with a bidirectional 1st order low-pass Butterworth filter with a cut-off frequency of 15 Hz (Kristianslund, Krosshaug, & van den Bogert, 2012). Ankle and knee joint angles was used in combination with shank length to estimate length of the gastrocnemius and soleus muscle tendon units based on regression equations for GM and SOL respectively (Hawkins & Hull, 1990). Estimation of the length of the elastic elements was calculated as described by (Fukunaga et al., 2001), subtracting the vertical fascicle length from MTU length.

Ultrasound recordings were imported to the analysis software "Ultratrack", where fascicle length and pennation angle were analyzed using a semi-automated tracking algorithm (Farris & Lichtwark, 2016) The length between the fascicle insertions to the superficial and deep aponeuroses was used to define fascicle length under the assumption that fascicle trajectory is linear. Linear extrapolation was employed to estimate fascicle length if the fascicle exceeded the ultrasound image. The angle between fascicles and the aponeurosis was used to define pennation angle. To calculate architectural gear ratio, the change in muscle length from touchdown to toe off was estimated from fascicle length and pennation angle by equation: *Muscle length = Fascicle length · cos (pennation angle).* Muscle thickness was also estimated from fascicle

length and pennation angle by equation: *Muscle thickness = fascicle length · sin (pennation angle).* Tracking of fascicles was attempted to be performed automatically, but a frame rate of 80Hz and shifts in plane of ultrasound imaging as a result of muscle bulging did not allow for this. Thus, manual correction of fascicle and aponeurosis tracking, frame-by-frame, was performed for all subjects. Modification of the tracking software was made, allowing the examiner to use keyboard buttons instead of the mouse to choose manual correction of upper- or lower fascicle insertion. This enabled the examiner to keep the eyes focused on the area of interest when correcting, potentially increasing the accuracy of tracking.
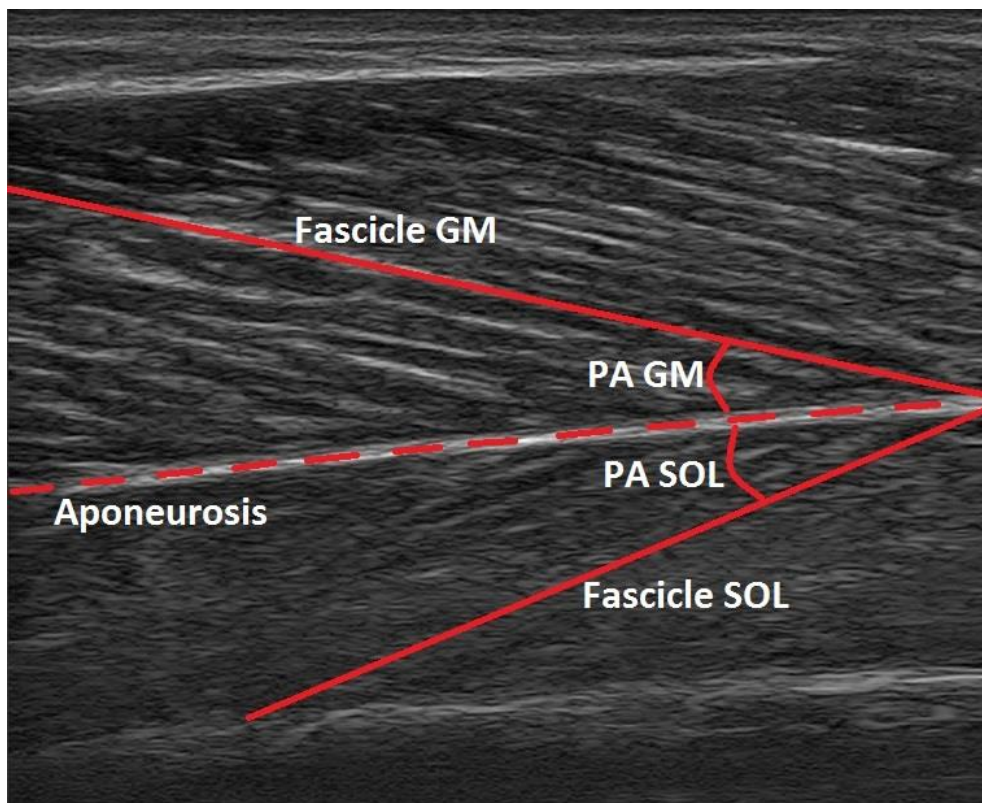
Figure 13

Ultrasound imaging of the gastrocnemius (GM) and soleus (SOL) muscles and aponeurosis. PA = pennation angle

Data reduction and statistical analysis

Analysis of changes in variables of interest during running was performed over the entire period of the stance phase (touchdown to toe off), or at relevant events/periods of stance. Events of interest include maximal values of length, velocity and moment. Periods of interest consist of sub-phases corresponding to lengthening and shortening of the MTU and elastic elements (EE) during stance. All time series data were resampled over 101 points. After visual inspection of variables of interest, step cycles that were strongly deviating from the pattern of most cycles were excluded. On average for all subjects, 8.8 out of 9.2 step cycles were included for analysis.

A repeated-measures, two-way ANOVA with factors time of testing (pre - post training) and group (training group - control group) was used to test differences in kinematics, joint moments, , fascicle behavior of the GM and SOL (length, pennation angle and velocity), MTU (length and velocity) and EE (length and velocity). In tests where significant main effects or time by group interactions were found, Sidaks post hoc test was employed. Statistical significance was set to P < 0.05. Results are presented as mean $\pm$ standard deviation in the text.

Results

Maximal voluntary contraction (MVC), tendon stiffness and strain

After the 10-week training intervention, the TG showed a significant increase in maximum voluntary ankle plantarflexion joint moment of ~15% ($P = 0.0013$), while the control group showed no change pre to post intervention. Achilles tendon stiffness for the TG increased significantly by ~18% ($P = 0.0009$), while the control group showed no change. AT strain decreased by ~9% for the TG, but the results did not reach statistical significance ($P = 0.1764$). The CG showed no change in AT strain (figure 14, table 1).
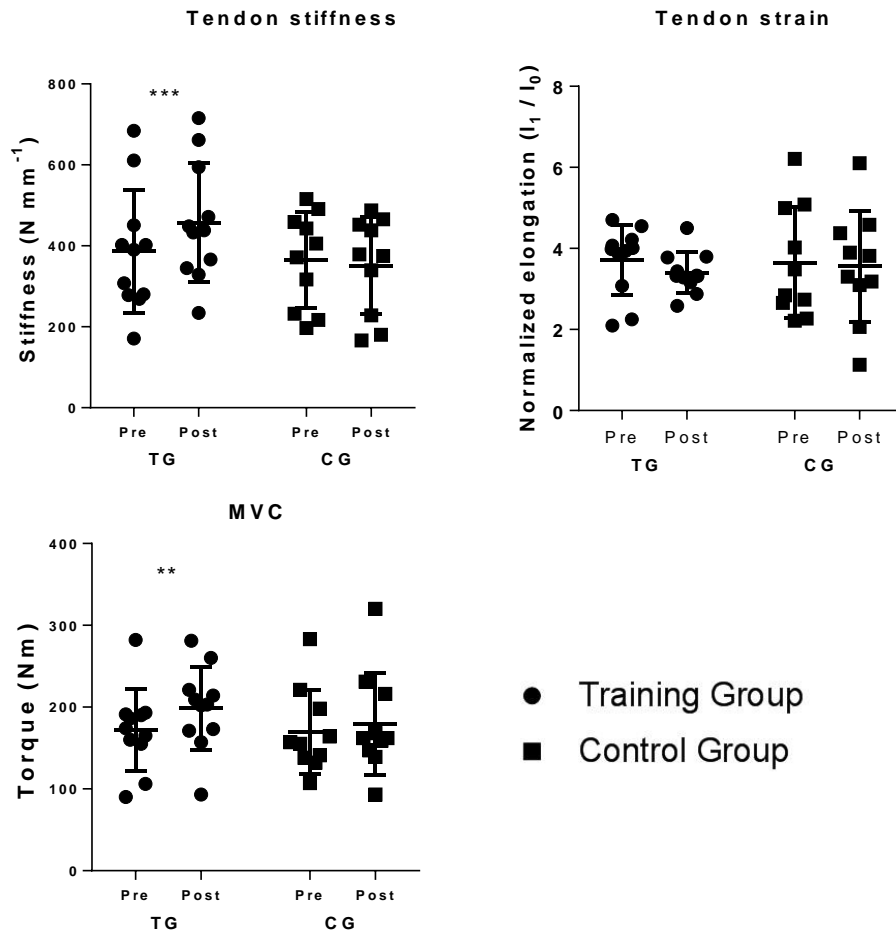

Figure 14

Values pre and post intervention for Achilles tendon stiffness (top-left), Achilles tendon strain (top-right) and maximal voluntary contraction (MVC) of the plantarflexors (bottom-left).

Table 1:

Mechanical properties of the muscle tendon unit determined during maximal and ramp contractions.

	Training group		Control group	
	Pre-training	Post-training	Pre-training	Post-training
Max. PF moment (Nm)	172 ± 50.0	198 ± 50.7**	169.6 ± 48.9	179.7 ± 59.2
AT stiffness (N/mm)	386.1 ± 152.8	457 ± 146.9***	364.8 ± 118.0	351.2 ± 119.9
AT strain (l_1/l_0)	$3,71 \pm 0,86$	$3,40 \pm 0,50$	$3,36 \pm 1,22$	$3,22 \pm 1,25$

Values are mean $\pm$ SD

max. maximum value

PF = Plantar flexion, AT= Achilles tendon

* Significantly different from pre-training value (p<0.05)

** Significantly different from pre-training value (p<0.01)

Kinematics, joints moments and ground reaction force

No significant change in either group was found in running kinematics after training with regard to ankle dorsiflexion and plantarflexion, knee flexion and extension and hip extension during stance (figure 15). Worth noting, the TG showed a trend of greater dorsiflexion of the ankle from touchdown to the point of peak dorsiflexion ($P = 0,0792$), while the CG showed no change in ankle kinematics (figure 15).

Neither the peak joint moments of the ankle, knee and hip (figure 15), nor the peak ground reaction force (figure 16, table 2) or impulse (table 2) of either group show any change from pre to post intervention.
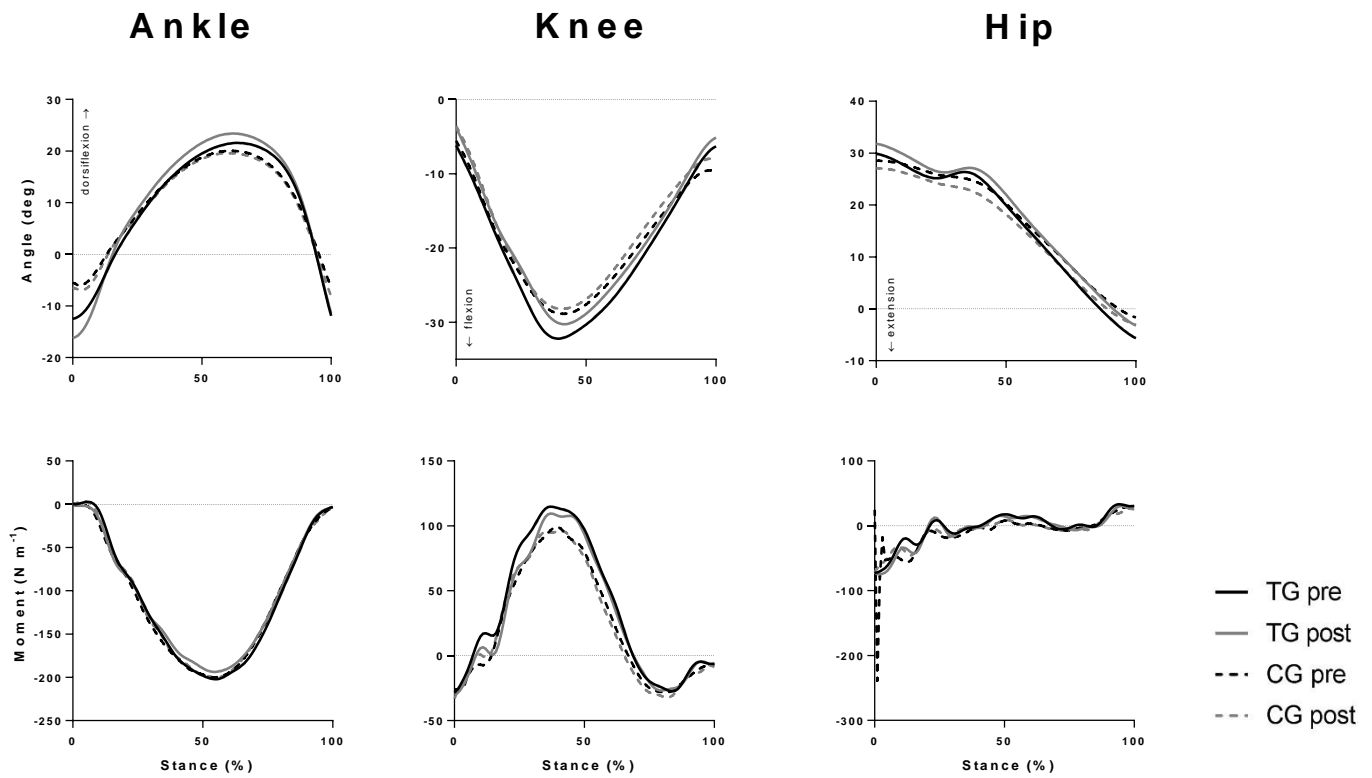
Figure 15

Average values of the ankle, knee and hip joint angles (top) and moment (bottom) during the stance phase of running before (pre) and after (post) the training intervention for the training group (TG) and control group (CG). The horizontal axis is normalized to the stance phase, where 0 % corresponds to touchdown and 100 % to take-off. For the ankle joint angle, positive values correspond to a dorsiflexed position and negative values to a plantar flexed position. For the knee, positive values correspond to an extended position and negative values to a flexed position. For the hip, positive values correspond to a flexed position and negative values to an extended position.
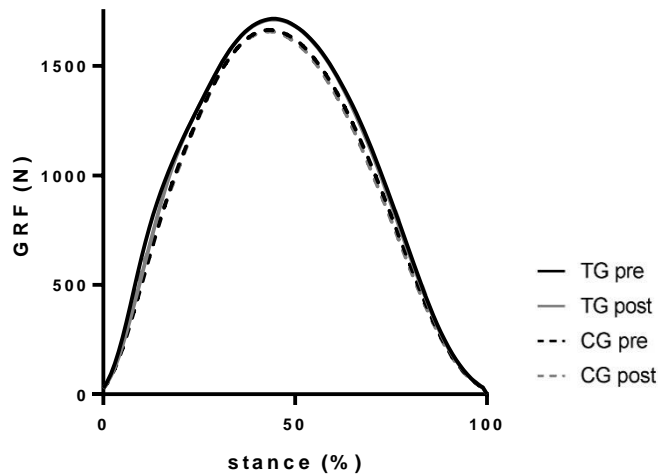
Figure 16

Average values of Ground Reaction Force (GRF) during the stance phase of running before (pre) and after (post) the training intervention for the training group (TG) and control group (CG). The horizontal axis is normalized to the stance phase, where 0 % corresponds to touchdown and 100 % to take-off.

Table 2

Peak ground reaction force and impulse during stance.

	Training group		Control group	
	Pre-training	Post-training	Pre-training	Post-training
Peak GRF (N)	1721 ± 302	1719 ± 279	1670 ± 132	1659 ± 137
Impulse (Ns)	253.3 ± 40.2	251.4 ± 37.4	261.1 ± 48.3	266.0 ± 41.3

Values are mean $\pm$ SD

GRF = Ground Reaction Force

* Significantly different from pre-training value (p<0.05)

** Significantly different from pre-training value (p<0.01)

Foot segment centre of pressure

A possible change in running strategy by an anterior shift of the point of force application (PFA) was tested by examining the position of the centre of pressure in the foot segment. The results showed no change pre to post intervention (Figure 17).
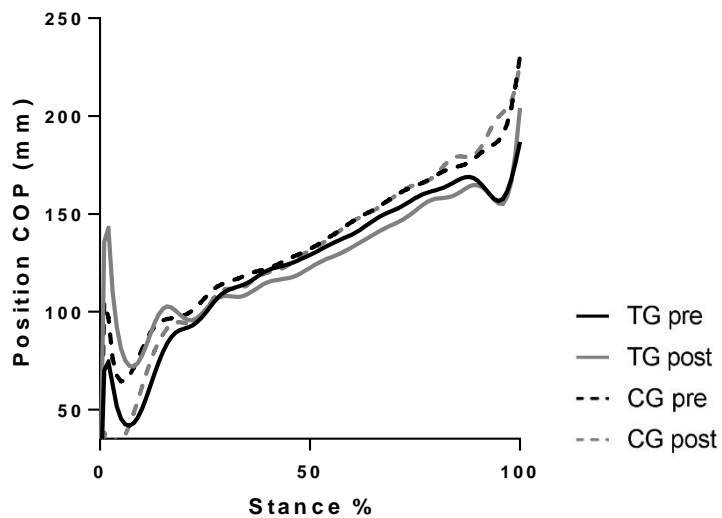
Figure 17

Average values of position of centre of pressure (COP) during the stance phase of running for running before (pre) and after (post) the training intervention for the training group (TG) and control group (CG). The horizontal axis is normalized to the stance phase, where 0 % corresponds to touchdown and 100 % to take-off.

Muscle tendon unit behavior

The extent of lengthening and shortening as well as maximal length of the GM and SOL MTU showed no significant change after the intervention. Neither GM nor SOL MTU velocity showed significant change in velocity during lengthening or shortening. However, a trend of increased lengthening was observed for both the GM (P =0.1330) and SOL (P =0.1052) MTU (figure 18, table 3).

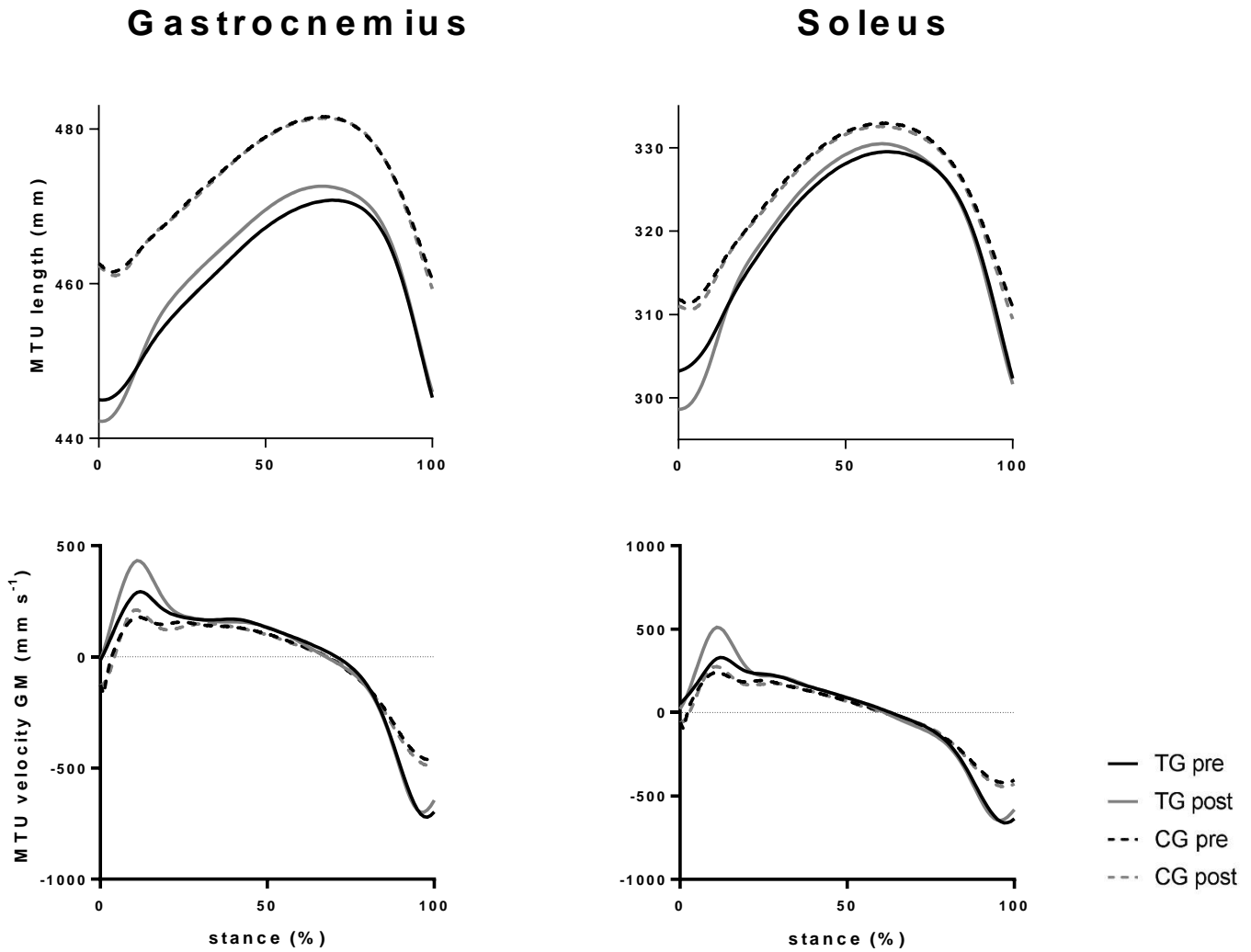# Gastrocnemius

Soleus

Figure 18

Average values of muscle tendon unit (MTU) length (top) and velocity (bottom) during the stance phase of running before (pre) and after (post) the training intervention for the training group (TG) and control group (CG). The horizontal axis is normalized to the stance phase, where 0 % corresponds to touchdown and 100 % to take-off.

Table 3

Muscle-tendon unit behavior during the stance phase of running

	Training group		Control group	
	Pre-training	Post-training	Pre-training	Post-training
GM MTU stretch (mm)	25.6 ± 10.3	30.5 ± 8.7	19.2 ± 7.4	18.6 ± 7.6
GM MTU shortening	15.2 ± 4.2	17.5 ± 6.2	12.4 ± 10.4	9.0 ± 5.9
SOL MTU stretch (mm)	25.8 ± 10.1	31.6 ± 8.9	21.5 ± 7.2	21.6 ± 7.2
SOL MTU shortening	16.6 ± 3.9	19.2 ± 6.4	19.2 ± 10.2	17.5 ± 6.4

Values are mean ± SD

max. maximum value

GM = Gastrocnemius, SOL= Soleus, MTU = muscle-tendon unit

* Significantly different from pre-training value (p<0.05)

** Significantly different from pre-training value (p<0.01)

Fascicle behavior

No significant change was found in resting fascicle length of the TG GM. However, an increase in resting pennation angle in the TG GM was found post intervention ($P = 0.0481$). No change in fascicle length or pennation angle was observed for the CG (table 4).

For either group (TG and CG) or muscles (GM and SOL) no significant change in fascicle shortening amplitude during stance was observed (figure 19, table 4).

There was not observed any significant change in both mean and maximum shortening velocity of fascicles after the training period for both the GM and SOL muscles in both the TG and CG (figure 19).

Mean pennation angle showed no change for either group or muscles. However, the TG showed a significant increase in GM pennation angle change from touchdown to toe off ($P = 0.0073$), while no significant change was observed for SOL. No significant changes in pennation angle for either muscle were observed in the CG (figure 19, table 4).

In the TG, architectural gear ratio of the GM increased, shown by an increase in ratio of Δmuscle length/Δfascicle length ($P = 0.0151$), while no change in architectural gear ratio was found for

SOL (figure 20). Mean muscle thickness or change in thickness (touchdown-toe off) did not show any change for either group (TG and CG) or muscle (GM and SOL) (table 5).

Gastrocnemius   Soleus
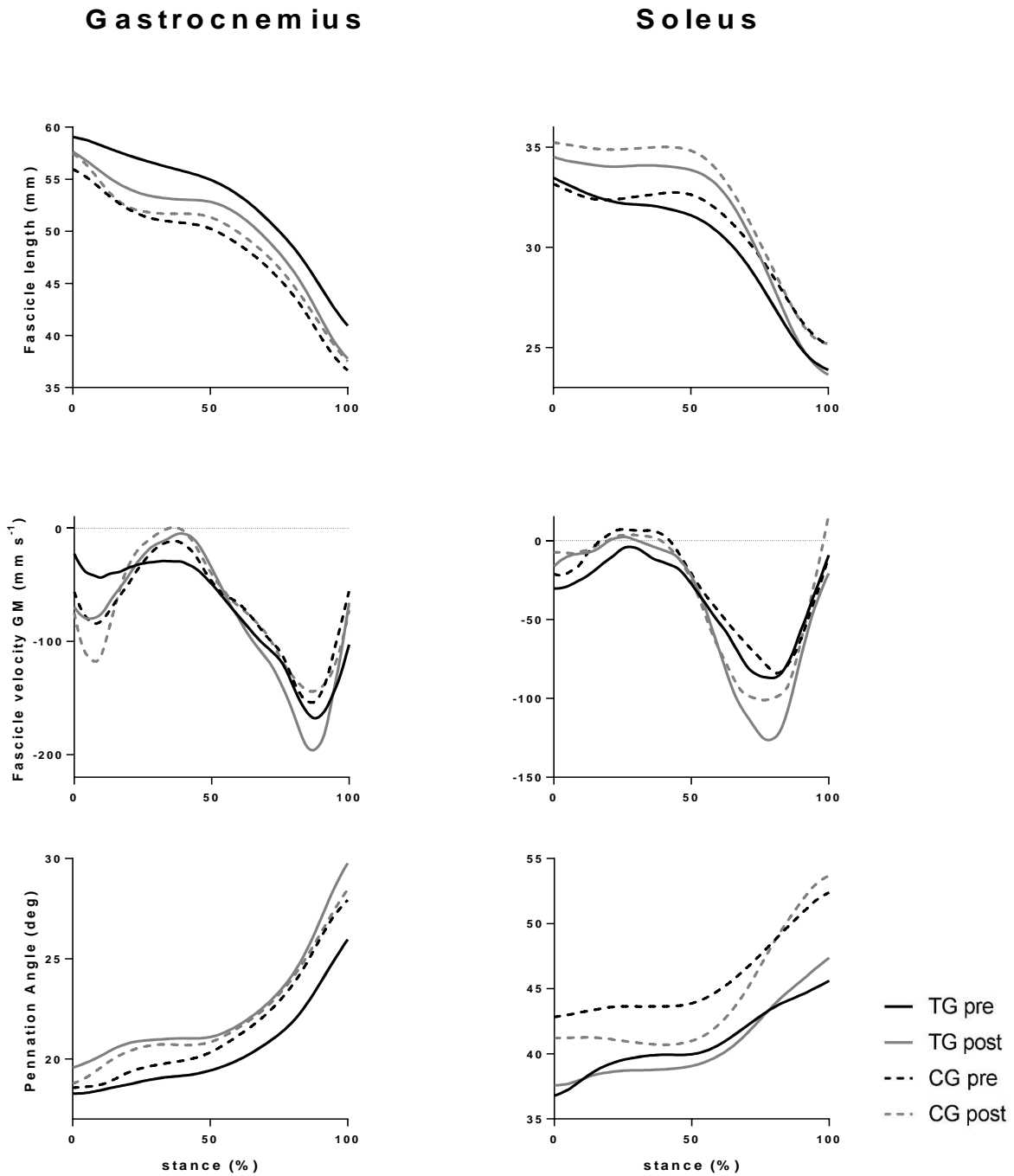
Figure 19

Figure 19

Average values of fascicle length (top), velocity (middle) and pennation angle (bottom) during the stance phase of running before (pre) and after (post) the training intervention for the training group (TG) and control group (CG). The horizontal axis is normalized to the stance phase, where 0 % corresponds to touchdown and 100 % to take-off.

Table 4

Resting fascicle length and pennation angle and fascicle behavior during the stance phase of running. Change is measured as the difference between values at touchdown and toe off.

	Training group		Control group	
	Pre-training	Post-training	Pre-training	Post-training
Resting FL GM (mm)	89.2 ± 13.4	90.7 ± 16.1	84.2 ± 10.3	84.1 ± 10.4
Resting PA GM (˚)	18.1 ± 1.8	19.0 ± 2.1*	18.4 ± 1.1	18.3 ± 1.7
GM FL shortening (mm)	16.2 ± 4.2	18.2 ± 2.2	18.45 ± 4.7	18.6 ± 4.1
SOL FL shortening (mm)	9.1 ± 2.5	10.4 ± 2.5	7.9 ± 2.8	9.8 ± 1.9
Δ GM PA (˚)	5.9 ± 3.5	9.2 ± 2.0**	9.0 ± 3.2	8.9 ± 2.1
Δ SOL PA (˚)	8.3 ± 3.1	9.2 ± 4.1	9.5 ± 3.0	12.1 ± 3.7

Values are mean ± SD

max. maximum value

GM = gastrocnemius, SOL= Soleus, FL= Fascicle length, PA = pennation angle

* Significantly different from pre-training value (p<0.05)

** Significantly different from pre-training value (p<0.01)

AGR from length GM

AGR from length SOL
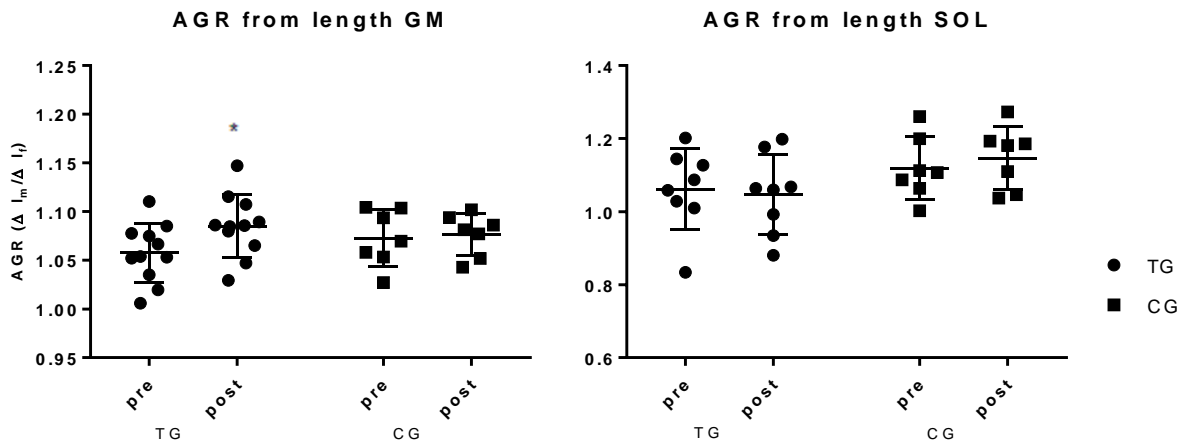
Figure 20

Values of architectural gear ratio (AGR) estimated from change in muscle and fascicle length from touchdown to toe off.

Table 5

Mean muscle thickness and change in muscle thickness from touchdown to toe off

	Training group		Control group	
	Pre-training	Post-training	Pre-training	Post-training
Mean MT GM (mm)	18.2 ± 3.3	19.0 ± 3.0	17.5 ± 4.2	18.0 ± 3.1
MT change GM (mm)	0.8 ± 1.1	0.6 ± 0.9	0.6 ± 0.7	0.3 ± 0.5

Values are mean ± SD

max. maximum value

MT = muscle thickness, GM = gastrocnemius

* Significantly different from pre-training value (p<0.05)

** Significantly different from pre-training value (p<0.01)

Elastic element behavior

Elastic elements (EE) showed no significant change in extent of lengthening or shortening for either group (TG and CG) in both muscles (GM and SOL). However, a trend of increased lengthening was observed for both the GM ($P = 0.0990$) and SOL ($P = 0.1528$) EE after the training intervention (figure 21, table 6).
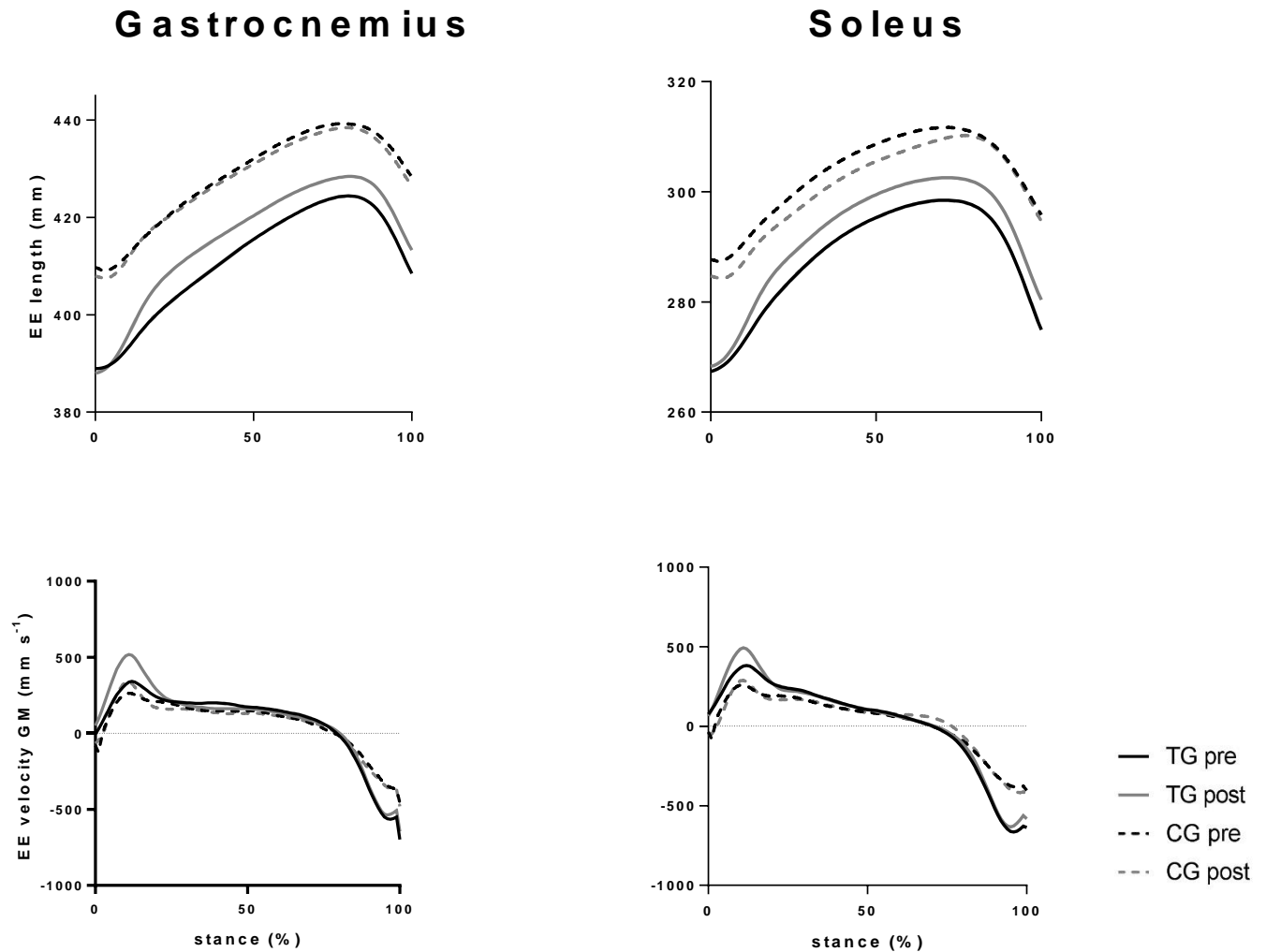
Figure 21

Average values of elastic element (EE) length (top) and velocity (bottom) during the stance phase of running before (pre) and after (post) the training intervention for the training group (TG) and control group (CG). The horizontal axis is normalized to the stance phase, where 0 % corresponds to touchdown and 100 % to take-off.

Table 6

Elastic element behavior during the stance phase of running.

	Training group		Control group	
	Pre-training	Post-training	Pre-training	Post-training
GM EE stretch (mm)	32.3 ± 10.4	37.5 ± 7.4	27.3 ± 6.9	26.2 ± 8.6
GM EE shortening (mm)	4.6 ± 4.0	5.4 ± 4.9	6.1 ± 6.0	3.3 ± 5.5
SOL EE stretch (mm)	30.1 ± 10.0	34.2 ± 8.7	23.6 ± 7.2	23.0 ± 7.0
SOL EE shortening (mm)	11.6 ± 5.0	11.4 ± 6.2	8.4 ± 9.9	2.8 ± 5.6

Values are mean ± SD

max. maximum value

GM = gastrocnemius, EE = Elastic elements

* Significantly different from pre-training value ($p < 0.05$)

** Significantly different from pre-training value ($p < 0.01$)

Discussion

The aim of the present study was to investigate whether increased Achilles tendon stiffness induced by explosive isometric resistance training of the plantar flexors affects the behavior of the GM and SOL fascicles during running. An increase in tendon stiffness would result in less strain at a given force acting on the tendon. Given that muscle fascicles of the GM and SOL are continuously shortening while the TS MTU is lengthening, less strain of the tendon was hypothesized to result in a reduced shortening of the muscle fascicles during lengthening of the MTU, as well as reduced contraction velocity of the muscle fascicles during stance.

After the 10 week training intervention, a significant increase of 18% in Achilles tendon stiffness and a significant increase of 15% in the maximum plantarflexion muscle strength were observed (figure 13). However, no significant change was observed for contraction velocity of the GM and SOL fascicles, nor shortening amplitude of the GM and SOL fascicles, rejecting our hypothesis (figure 18).

Effectiveness of the training intervention

Strength and architecture

Muscle fascicle length has previously been shown to increase with dynamic resistance training (Alegre, Jimenez, Gonzalo-Orden, Martin-Acero, & Aguado, 2006). Resting length of the muscle fascicles did not increase after training in this study. This was expected, as the contractions performed in the exercise intervention was executed explosively and lasted for only 1 second, limiting time under tension.

However, an increase in resting pennation angle was found after training (table 4). This is consistent with previous studies (Aagaard et al., 2001) employing measurements of pennation angle by ultrasound following an intervention of intense resistance training. An increase in pennation angle implies an increase in physiological cross sectional area in proportion to anatomical cross sectional area resulting in an increased strength in proportion to muscle volume (Aagaard et al., 2001).

The AT is attached to the GM and SOL muscles, and inducing adaptations in the tendon will inevitably also affect the muscles. The aim being to examine how tendon stiffness affects fascicle behavior, it is of importance to minimize muscle adaptation. With the exercise protocol employed in this study we were able to limit changes in fascicle length. However, the muscle has undergone distinct adaptations as pennation angle and muscle thickness increased after training. The observed effect of training on the muscle alone increased strength significantly, but not the fascicle length operating range or velocity during running. The muscle adaptations observed in the current study will likely have an influence on fascicle behavior, but should be limited due to small change scores.

Tendon stiffness

The design of the exercise, aiming to minimize muscle adaptations will likely also have limited tendon adaptations. Tendon stiffness has been shown to increase as early as 6-8 weeks after systematically increased mechanical loading of the Achilles tendon (Wiesinger et al., 2015). Studies with training interventions of similar duration as ours have achieved an increase in AT stiffness greater than the current study (Arampatzis et al., 2007; K. Kubo, Ikebukuro, Yata, Tsunoda, & Kanehisa, 2010a), which could be the result of different exercise protocols. A meta-analyses of human tendon adaptation in response to mechanical loading found that high loading intensities are more effective to induce adaptive responses in tendinous tissue as compared to lower intensities, while the type of contraction performed by the muscle does not seem to affect the extent of adaptive responses (Bohm, Mersmann, & Arampatzis, 2015). Dynamic as well as isometric contractions imposing a large strain magnitude on the tendon would therefore seem to be best suited to achieve a large extent of adaptation responses of the tendon. With the aim of limiting adaptation responses of the muscles, the exercise performed by the subjects in this study involved isometric contractions at anatomical position to avoid an increase in number of sarcomeres in series. Contractions lasted for a limited duration of 1 second to minimize hypertrophy of the muscle by liming time under tension. The comparably small increase in tendon stiffness is likely a consequence of the exercise exerting an insufficient time under

tension and strain magnitude on the tendon (Arampatzis et al., 2007), which may be needed to achieve a greater increase in tendon stiffness than achieved in the current study.

Running kinematics

In line with previous studies examining running before and after an intervention of strength training of the plantar flexors (Albracht & Arampatzis, 2013), kinematics and of the ankle, knee and hip did not show any significant difference pre to post intervention. However, a trend of greater dorsiflexion of the ankle in the first ≈25% of stance was found, implying a possible change in running strategy after the training intervention.

The trend of increased dorsiflexion was thought possibly to be the result of an anterior shift of the point of force application (PFA). A change in running strategy involving an anterior shift of the PFA has previously been speculated to occur after a similar exercise intervention (Albracht & Arampatzis, 2013). To further examine this, the position of the centre of pressure of the foot segment was examined, but no change was found between pre and post intervention tests in the present study (figure 16).

Fascicle behavior

In line with previous studies (Lichtwark et al., 2007), we find muscle fascicles to show a continuous shortening throughout the stance phase. This implies that the energy generated by the muscle is stored within the elastic elements during the first phase of stance, as the MTU was found to be lengthening for the first ≈75% of stance (figure 15). As the MTU and elastic elements shorten rapidly in the latter ≈25% of stance, elastic energy is returned to the MTU, reducing the demand for fascicle shortening and contraction velocity.

The force generating capacity of a muscle is affected by the length at which the muscle fascicles are generating force. The length of the muscle fascicle determines the extent of overlap between actin and myosin cross-bridges, and too much or too little overlap results in a reduction of the force producing capacity of the muscle. Increased shortening amplitude of muscle fascicles

would imply the muscle fibers have to generate force at shorter lengths, where capacity for force production is reduced. Contraction velocity of the muscle fascicles also influences the force producing capacity of the muscle, with increasing velocities reducing the capacity for producing force. The hypothesized reduction in fascicle shortening amplitude and contraction velocity would lead to better conditions for the muscle to produce force. However, as we find both the GM and SOL muscles to have an unchanged shortening amplitude and contraction velocity, they are left under the same conditions for force production as before the training intervention.

Analysis of fascicle behavior of the SOL muscle proved to be especially challenging. Visualization of especially the deeper lying part of the SOL muscle as well as the deep aponeurosis was poor in several subjects and we experienced prolonged periods of having close to no view of muscle fascicles. As a result, the SOL muscle in three subjects of the TG was excluded. Nevertheless, SOL fascicle behavior is consistent with that of the GM after training, strengthening the evidence that no change in shortening amplitude or velocity occurred in either muscle.

Although fascicle behavior did not change in the way expected, a change did seem to occur as we observed a significant increase in pennation angle change in the GM muscle, while no change was found for SOL. Pennation angle changes towards a more obtuse angle during contraction and contributes to muscle shortening. A greater change in pennation angle while shortening amplitude of the muscle fascicles remains unchanged means that the muscle shortens to a greater extent.

Architectural gear ratio

A greater shortening of the muscle as a result of an increase in pennation angle change while shortening amplitude of the fascicles remain unchanged implies an increased architectural gear ratio. Changes in architectural gearing within the same muscle has been examined previously (Azizi et al., 2008), but has to our knowledge never been examined in running. Length of the whole GM muscle belly was not directly measured in this study. The method of estimating muscle shortening used in the current study is subject to uncertainty as the changes in muscle length are not directly measured, but rather based on changes in fascicle length and pennation

angle during contraction. However, muscle shortening is calculated as the difference of the estimated muscle length at touchdown and toe off and should provide a good indication of changes in muscle shortening. The ratio of the change in muscle length/fascicle length during stance was found to be increased post intervention for the TG GM, indicating an increased gear ratio. As reported by Azizi et al., 2008, a change in architectural gear ratio is associated with differences in dynamic muscle shape changes. Therefore, we estimated muscle thickness from fascicle length and pennation angle throughout the stance phase. However, no difference in mean thickness or change in thickness from touchdown to toe off was found (table 5).

The higher strength of the plantar flexors post intervention could be speculated to result in the muscles operating at a lower percentage of their maximal force producing capacity. The muscle could also contract faster as pennation angle change increased while fascicle shortening amplitude and changes in muscle thickness remains unchanged. Force dependency of architectural gear ratio has previously only been shown with absolute force changes. It could be speculated that a faster contraction at a lower relative load could also lead to an increase in architectural gear ratio.

Elastic element strain

Because running kinematics remained unchanged, the unaffected fascicle shortening and velocity could mean that the increase in tendon stiffness did not notably affect tendon strain during running. Consistent with a trend of more dorsiflexion, a trend of increased lengthening of the GM and SOL MTU (figure 17) and EE (figure 20) was observed. Considering this, and that the muscle shortens to a greater extent, EE strain during running may have increased after training. Increased EE strain would imply a greater storage of elastic energy. An increase in EE strain and greater storage of elastic energy would be expected to be followed by a greater return of elastic energy in the late stance phase that would be reflected by an increased shortening of the MTU and EE. This was however not observed.

The reason for a trend of increase in EE strain and elastic energy storage cannot be assessed from this study. In addition to the free AT, the EE also consist of the aponeurosis of the muscle and the proximal tendon of the GM. The aponeurosis has been shown to experience less stretch than

the free AT during plantar flexion contractions (Finni, Hodgson, Lai, Edgerton, & Sinha, 2003; Magnusson et al., 2003), possibly owing to different material properties or as a consequence of transverse tension imposed by muscle bulging (Farris, Trewartha, McGuigan, & Lichtwark, 2013). Thus, changes in the force production of the muscle might affect functional aponeurosis stiffness. This has been shown, as aponeurosis of muscles increase stiffness with increased force production of the muscle (Azizi & Roberts, 2009). As higher strength of the muscles after training could lead to the muscle generating force at a lower relative load, it could be speculated that functional stiffness of the aponeurosis decreased post intervention, explaining an increased strain of the EE.

Increased strain of the EE could also be explained by a larger force production of the muscle. A larger force production could be reflected by either increased ground reaction forces, or an increase in ankle moment. However, no change in neither GRF, impulse or ankle moment was found. Had the increased dorsiflexion found in the first phase of stance been significant, this would have implied a larger external moment arm of the ankle. An increase in the external moment means the mechanical advantage would be reduced and a larger muscle force of the plantar flexors would be required to produce the same ankle moment. However, this would imply a lower GRF, which was found to remain unchanged. GRF measurements of running made with a force-plate instrumented treadmill lose validity when landing strategies are not consistent (Kluitenberg, Bredeweg, Zijlstra, Zijlstra, & Buist, 2012). Landing strategies of the subjects was only observed visually, making for uncertainty of GRF measurement comparisons in the current study. As opposed to platforms mounted in the laboratory floor, force plates incorporated in treadmills are subject to more noise from close lying moving parts. A cut-off frequency of 25 Hz was applied to GRF measurements as a consequence of noise stemming from vibrations from the belt of the treadmill, which could affect the validity of GRF measurements.

Although GRF measurements have potential sources of error, the fact that all subjects ran barefoot is thought to limit variations in landing strategy. The common landing strategy running barefoot, even by pronounced heel-strikers when shod, is a forefoot strike. Supported by visual inspection, all subjects landed using a forefoot strike. When compared across subjects with a

consistent landing strategy, GRF measurements have been shown to have a moderate to high validity (Kluitenberg et al., 2012).

Assuming GRF measurements are correct, and GRF remained unchanged after the intervention, a larger force production of the muscle would have been associated with an increased ankle moment. No change in ankle moment was observed, but the estimation of ankle moment is subject to uncertainty. Strong evidence is present that human foot power contributes meaningfully to running, by a complex interaction of tendinous tissue and muscles of the foot, both absorbing and generating mechanical power. Typically, as well as in the present study, the entire foot is modeled as a single rigid segment neither absorbing nor generating mechanical power. Treating the foot as a single rigid segment can be misleading to our understanding of foot function, and further, calculations of ankle joint moment ignore the contribution and complexity of the human foot. It is therefore possible that changes in ankle joint moment pre to post training intervention could have occurred. Using simplified modelling of the foot could possibly leave us unable to detect it (Zelik & Honert, 2018).

Limitations

The in vivo measurements employed in the current study present limitations on several fronts that should be considered objectively.

Fascicle tracking

The length changes of muscle fascicles often determine their function and effectiveness of performing the task at hand during movement, laying the basis of the interest in examining fascicle behavior. The method of fascicle tracking employed in the present study involves drawing a line parallel to the visualized fascicle in the mid-muscle belly is drawn from its insertion at the superficial and deep aponeurosis. Analyzing fascicle behavior of a whole muscle using this method is assuming that fascicles experience the same strain along its entire length. However, the strain along a muscle fascicle has been shown to vary along its length (Pappas, Asakawa, Delp, Zajac, & Drace, 2002). Previous modeling studies have shown that such variation likely results from curvature of the fascicle and variations in mechanical properties of

surrounding tissue (Blemker, Pinsky, & Delp, 2005). In addition to longitudinal changes in length, fascicles of pennate muscles undergo changes in orientation reflected by a change in pennation angle, affecting fiber strain. In pennate muscles like the GM and SOL examined in this thesis, variations in muscle architecture within regions of the muscle introduces an additional factor resulting in further variations in fiber strain within the muscle. Further development of software used for tracking of fascicles, taking variable strain into account, is needed for future studies to ascertain that tracking of muscle fascicles is representative of the fascicle behavior of the muscle.

Soleus fascicle tracking

In the current study, an addition to measurements of the GM muscle performed by earlier studies is examining the fascicle behavior of the SOL muscle. The GM is a biarticulate muscle acting over both the ankle and knee joint, and could be affected by changes in properties in the proximal part of the muscle, crossing the knee joint, which is not examined in this study. Examining fascicle behavior of the soleus muscle, which only acts across the ankle joint, could therefore with a higher certainty be attributed to the changes in tendon and muscle properties imposed by the exercise protocol. However, ultrasound imaging of the soleus muscle was more variable and of poorer quality than those of the GM. Including the soleus muscle also meant making compromises with regard to the focus area of ultrasound imaging, sometimes limiting the visibility of the soleus muscle fascicles and deep aponeurosis. Limited visibility and periods of practically no view of muscle fascicles made for more challenging tracking, with higher potential sources of error. Further development of ultrasound equipment is required for future studies to be able to properly visualize the SOL muscle, and allow for reliable analysis of SOL fascicle behavior.

Ultrasound

The current study employs use of two dimensional ultrasound scanning that captures tendon deformation only in the longitudinal direction. The same ultrasound scanning is performed when scanning the GM an SOL muscles during running. Tendons and muscles are three dimensional structures that undergo deformation in all three dimensions. Not taking into account the three

dimensional structure and deformation of the tendon leads to an underestimation of tendon length and an overestimation of length changes (Seynnes et al., 2015). Concerning muscle scanning, we experienced that the frame rate of the ultrasound recording (80Hz) was insufficient for the employed software to consistently perform automatic tracking of the muscle fascicles. As the muscle bulges during contraction when running, the ultrasound transducer changes its position relative to the muscle belly. This results in a shift of the plane of scanning, making for difficulties tracking the same muscle fascicle throughout the step cycle. In several subjects, the field of view captured by the ultrasound transducer did not capture the entire fascicle and its insertion to the superficial and deep aponeurosis. This was experienced primarily in the latter part of the swing phase, where muscle fascicle length is at its extreme. In cases of incomplete scanning, linear extrapolation was used, assuming both fascicle and aponeurosis followed a straight line outside the field of view, making for potential sources of error estimating fascicle length. 3D ultrasound scanning, and a larger field of view of the ultrasound transducer is required for future studies to reliably analyze tendon and fascicle behavior and gain a better understanding their function and response to training.

Kinematics

This study performed non-invasive three dimensional motion analysis using markers attached directly to the skin using adhesive tape. The marker trajectories are recorded while subjects were running, and laboratory coordinates are estimated in each sampled instant of time. From markers attached to the ankle (medial and lateral malleolus), knee (medial and lateral condyle of the knee), and hip, the trajectory of the respective joint centres were calculated. Further calculations of moments and power are also based on the position of joint centres estimated by marker trajectories. However, recorded marker points cannot be considered stationary as the markers are attached to passive and active soft tissue that moves relative to underlying bony structures. When reconstructing marker positions, systematic and random errors can also occur. This highlights a limitation and potential sources of error in the present study, where we are describing running kinematics using non-invasive three dimensional motion analyses (Lucchetti, Cappozzo, Cappello, & Della Croce, 1998).

Conclusion

The intervention did not reduce fascicle shortening and contraction speed as expected from simple in series models. Although not in the way we expected, a change in fascicle behavior was observed after training, as GM pennation angle change increased. Increased pennation angle change and unchanged shortening amplitude of muscle fascicles means that the muscle shortens to a greater extent, resulting in an increased architectural gear ratio. Higher strength of the plantar flexors could mean the muscles are operating at a lower force relative to maximal force production, which could be speculated to promote fiber rotation and cause the increased architectural gear ratio. Considering the consistency of trends of increased dorsiflexion, lengthening of the MTU and EE accompanied by an increase in muscle shortening, EE strain may be increased, increasing utilization of elastic energy. An increase in utilization of elastic energy while fascicle shortening amplitude and velocity remains unchanged would be beneficial for running efficiency. Additional studies and further development of fascicle tracking software and ultrasound equipment are required to ascertain that muscle shortening, architectural gear ratio, and EE strain increased and if this is an effect of training.

Statement

Writing this thesis, I have been a part of a larger Ph.D. project. I entered the project a short time before the start of data collection and have not been a part of designing the test protocol nor the exercise intervention. I have contributed to all data collection. However, I have not participated in all data processing and analysis. My main task with regard to data analysis has been tracking of GM and SOL muscle fascicles and aponeurosis using the "Ultratrack" software. I have also performed data curation of all data collected except tendon stiffness data. I have however not been a part of data processing and analysis of tendon stiffness data nor kinematic and kinetic data in Visual 3D.

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Attachment 1: Approval by local ethics 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 v d ere 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

NIH NORGES IDRETTSHØGSKOLE

Besøksadresse Sognsveien 220, Oslo
Postadresse Pb 4014 Ullevå Stadion, 0806 Oslo
Telefon +47 23 26 20 00, postmottak@nih.no
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Attachment 2: Form of informed consent:

Rollen av muskel- og sene-egenskaper i den mekaniske funksjon av de menneskelige nedre ekstremitetene

NORGES IDRETTSHØGSKOLE

ROLLEN AV MUSKEL- OG SENE-EGENSKAPER I DEN MEKANISKE FUNKSJON AV DE MENNESKELIGE NEDRE EKSTREMITETENE

Dette er et spørsmål til deg om å delta i en forskningsprosjekt for 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.

HVA INNEBÆRER PROSJEKTET?

Isometriske kontraksjoner med dreiemomentmålinger kombinert med ultralyd vil bli brukt til å bestemme individuelle egenskaper ved akillessenene. Videre skal en kombinasjon av bevegelsesanalyse, reaksjonskrefter fra underlaget og ultralyd brukes til å analysere muskelfasciens atferd og seneforlengelse i løpet av dynamiske bevegelser. Bevegelsene skal være løp på tredemølle, repetitive hopp, knebøyhopp og fall-landinger. De valgte metodene har blitt brukt i flere tidligere studier.

I prosjektet vil vi innhente og registrere opplysninger om deg. Antropometriske og fysiologiske data vil bli samlet (høyde, vekt, segmentlengder av leggen, muskel fascikle og senens lengder). Dessuten skal kinetisk og kinematisk informasjonen bli innsamlet (reaksjonskrefter fra underlaget, leddvinkler).

MULIGE FORDELER OG ULEMPER

Hver deltaker får innsikt i vitenskapelige undersøkelser i et område som er relatert til deres idrettsinteresse. Dessuten har deltakerne muligheten å lære om optimaliseringen muskel-sene funksjonen gjennom deres resultater. Intervensjonsgruppen kan dra nytte av en profesjonell treningsplan, samt veiledet trening i intervensjonsperioden.

Risikoen for skader av deltakerne er lik en normal risiko for fysisk aktivitet. Implikasjonen av målemetoder endrer ikke bevegelsesmønsteret og øker dermed ikke risikoen for skader. Oppsummert er denne risikoen ansett å være meget lav. Dersom skader mot formodning skulle oppstå, er forholdsregler tatt for å gi deltakerne førstehjelp.

Rollen av muskel- og sene-egenskaper i den mekaniske funksjon av de menneskelige nedre ekstremitetene

FRIVILLIG DELTAKELSE OG MULIGHET FOR Å TREKKE SITT SAMTYKKE

Det er frivillig å delta i prosjektet. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke. Dette vil ikke få konsekvenser for din videre behandling (Siste setning fjernes dersom deltakeren ikke rekrutteres i kraft av å være pasient). Dersom du trekker deg fra prosjektet, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner. Dersom du senere ønsker å trekke deg eller har spørsmål til prosjektet, kan du kontakte Amelie Werkhausen, telefonnummer +4723262324, amelie.werkhausen@nih.no.

HVA SKJER MED INFORMASJONEN OM DEG?

Informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Du har rett til innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert.

Alle opplysningene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger gjennom en navneliste.

Prosjektleder har ansvar for den daglige driften av forskningsprosjektet og at opplysninger om deg blir behandlet på en sikker måte. Informasjon om deg vil bli anonymisert eller slettet senest fem år etter prosjektslutt.

FORSIKRING

Norges idrettshøgskole dekker utgifter for eventuelle skader deltakerne måtte pådra seg i tilknytning til studiet.

Rollen av muskel- og sene-egenskaper i den mekaniske funksjon av de menneskelige nedre ekstremitetene

SAMTYKKE TIL DELTAKELSE I PROSJEKTET

JEG ER VILLIG TIL Å DELTA I PROSJEKTET

Sted og dato   Deltakers signatur

                                                  _____
                                                  Deltakers navn med trykte bokstaver

Jeg bekrefter å ha gitt informasjon om prosjektet

Sted og dato   Signatur

                                                  _____
                                                  Rolle i prosjektet