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Differences in Properties of the Triceps Surea Muscle Tendon Unit in Professional Dancers and Active Controls

A cross-sectional sudy with emphasis on stretching exercise

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MASTER THESIS

Differences in Properties of the Triceps Surea Muscle Tendon Unit in Professional Dancers and Active Controls

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Preface

Merci beaucoup, mange tak, tusen takk!

First and foremost owe my most sincere gratitude to my first supervisor Olivier Seynnes. You sparked my interest for this project in the first place! I thank you profoundly for your limitless support from start to finish of the present thesis. Without your proficient and competent advice, patience, knowledgeable insights and your expectations of high standard work, I would never have learned as much and I would not have been able to complete this thesis.

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Last, but not least I owe my immediate family, my grandparents and my darling fiancée and NIH colleague, Eirik Myhr Nossum a massive thanks for your support in all my endeavors and projects. I promise you will see a little more of me in the near future! Finally the guys at the office must be mentioned. Even Granerud, Eirik Myhr Nossum and Kristoffer Bergstrøm, even though you annoy me with all your “guy-ness” at times I have loved sharing an office with you and also getting to know you during our years of studying together. You made the long days and nights not only endurable, but also enjoyable.

Abbreviations

Abbreviation	What
AT	**A**chilles **T**endon
CC	**C**ontractile **C**omponents
CSA	**C**ross-**S**ectional **A**rea
ECM	**E**xtra**c**ellular **M**atrix
EMG	**E**lectro**m**yo**g**raphy
Free AT	**F**ree **A**chilles **T**endon
GM	**G**astrocnemius **M**edialis
GL	**G**astrocnemius **L**ateralis
GTO	**G**olgi **T**endon **O**rgan
IRC	**I**sometric **R**amp **C**ontraction
MTJ	**M**yo**t**endinous **J**unction
MTU	**M**uscle-**T**endon **U**nit
MVC	**M**aximal **V**oluntary **C**ontraction
PEC	**P**arallel **E**lastic **C**omponents
PH	**P**robe **H**older
PNF	**P**roprioceptive **N**euromuscular **F**ascilitation
ROM	**R**ange **o**f **M**otion
SEE	**S**eries **E**lastic **E**lements
SOL	**S**oleus
SR	**S**arcoplasmatic **R**eticulum
TIB	**Ti**bialis **A**nterior
US	**U**ltra**s**ound
VAS	**V**isual **A**nalogue **S**cale

Abstract

Purpose: The purpose of this thesis is to compare structural- and mechanical properties in the triceps surae muscle-tendon unit system (MTU) of professional dancers with >15 years of systematic stretching to a control group consisting of physically active subjects.
Method: 20 healthy subjects (dancers n=10, controls n=10) participated and one leg was tested. Ultrasound images (US) of muscle architecture was assessed, including muscle thickness, fascicle pennation angle and fascicle length. US images of tendon (CSA) area and tendon length were analyzed. The elongations of the Free Achilles tendon (free AT) and gastrocnemius medialis tendon (GM tendon) in ramped isometric plantarflexions up to the voluntary maximum were directly measured with ultrasound. Maximal dorsiflexion range of motion (ROM) and passive torque was measured in an isokinetic dynamometer. Young's modulus and stiffness was assessed.
Results: ROM was larger in dancers compared to controls ($P < 0.01$). Dancers displayed longer GM muscle fascicles (12.8 ± 0.6 mm) than controls (9.5 ± 0.3mm), and thicker GM than controls (Thickness: controls 17.7 ± 2 mm, dancers 21.7 ± 2 mm) with $P < 0.01$. Both free AT and GM tendons were longer in dancers (GM tendon: 220 ± 32 mm. free AT: 81.7 ± 33) compared to controls (GM tendon: 175 ± 19. Free AT: 55 ± 25) with $P < 0.01$. No difference was found in free AT CSA, Young's modulus or mechanical properties.
Conclusions: Dancers displayed different structural and morphological properties in the triceps surea MTU compared to controls. It is likely that the differences found in the present study results from substantial differences in stretching exercise volume, frequency and intensity. There were no differences in mechanical properties of the MTU.

KEYWORDS:
TENDONS, MUSCLES, STRETCHING EXERCISE, MUSCLE ARCHITECTURE, MECHANICAL PROPERTIES, DANCERS

1.0 Introduction

Joint range of motion (ROM) is one of many physical properties that contribute to a person's ability to execute a movement task in an appropriate manner. Thus, stretching is often considered as an integral part of any exercise program. Systematic stretching is commonly used in both professional sports and in recreational activities, because it leads to increased joint ROM (Toft, Espersen, Kalund, Sinkjær & Hornemann, 1989; Halbertsma, Ludwig & Gôeken, 1994; Kubo, Kanehisa, & Fukunaga, 2002a, 2002b; Nakamura, Ikezoe, Takeno & Ichihashi, 2011: Mizuno, Matsumoto, & Umemura, 2013; Wilson, Elliot & Wood, 1991). Moreover, many sport-specific movement tasks require a large muscle-tendon operating range for optimal performance. Examples are dance, gymnastics, rhythmic gymnastics, diving, Olympic weightlifting and figure skating. Thus, the muscles need to produce large forces at joint angle ranges that are hardly necessary in daily life. The current literature is incoherent regarding whether stretching leads to increased sports-performance and if stretching aids injury prevention (Herbert & Gabriel, 2002). It is known that stretching training increases ROM in healthy joints. In addition, current evidence shows that stretching also restores movement to an injured or limited joint (Zhao, Wu, Hwang, Ren, Gao, Gaebler-Spira, Zhang, 2011; Cipriani, Terry, Haines, Tabibnia, & Lyssanova, 2012). The ambiguous opinions about the effects of stretching may be due to the fact that the exact mechanisms for changes in joint ROM are not fully known. Mechanical-, material- and morphological properties of the muscle-tendon unit will have implications for the muscles' and/or tendons' ability to adapt to stretching exercise and possibly increase ROM. Interestingly, professional elite dancers practice systematic stretching of large volumes and high frequencies in their daily training. They start stretching from a very young age (5 years) and continue throughout their entire professional career. By examining the muscle tendon unit system (MTU) in dancers it may be possible to investigate whether tendon mechanical properties, muscle architecture, muscle- and tendon morphological properties are different compared to controls as a result of stretching.

2.0 Purpose and hypothesis

The purpose of this thesis is to compare structural and mechanical properties in the triceps surae MTU of elite professional dancers with >15 years of systematic stretching to a control group consisting of physically active subjects.

An increase in flexibility might be achieved by changes in the mechanical properties of the MTU and joint, and/or changes in structural properties of the MTU. The expectancy was that this the dancers would display mechanical, architectural and /or morphological differences in the MTU tissue when compared to a matched control group that had never undertaken stretching close this volume. Dancers were expected to display lower passive torque during passive dorsiflexion, and thus greater ankle joint flexibility than controls. A morphological difference in the MTU tissue in dancers could include greater muscle fascicle lengths than active controls. Previous animal studies argument for these possible outcomes (Warren, Lehmann, & Koblanski, 1976; Stolov, Weilepp, & Riddell, 1970; Tabary, Tabary, Tardieu, Tardieu, & Goldspink, 1972). In previous human long-term with stretching interventions of durations up to maximum 10 weeks increased ROM was recorded, without change in tendon stiffness (Halbertsma et al., 1994; Halbertsma et al., 1996; Magnusson et al., 1996b; Ben & Harvey, 2010; Weppler & Magnusson, 2010, 2010). Such relatively short intervention durations may not be sufficient to elicit changes in mechanical properties. Therefore, despite inherent limitations a cross-sectional study between subjects exposed to long-term stretching and controls can be justified.

Hypothesis 1:
When compared to the control group the MTU system of the dancers will display less passive resistance to stretch, and thus dancers possess larger maximal dorsiflexion ROM.

Hypothesis 2:
Differences in flexibility are reflected by adaptive differences in morphology, architecture and/or mechanical properties of the muscle and/or tendon. Such adaptations might be longer muscle fascicles.

3.0 Theory

3.1 Definitions and Terminology

3.1.1 Morphological properties

Morphological properties are variables that describe muscle or tendon dimensions, such as cross sectional area (CSA), volume or length. Mechanical properties refer to the characteristics and behavior of a material when forces are applied to it, e.g. when tissues are stretched passively. Mechanical properties are dependent on the geometry of a body. Such properties may for example be stiffness or compliance.
The molecular composition and organization of a tissue will determine the structure of the tissue. The mechanical properties of a tissue normalized to a body's dimension are the material properties. In this respect the material properties of a body refers to tissue construction and composition. (Wang 2006; Kjaer, 2006)

3.2 Range of Motion: Definition and limiting factors

Clinicians and practitioners commonly use the term *flexibility*, however in research and clinic many appear to use a variety of terms to characterize the ROM. ROM, or total ROM or maximal ROM describes the joint's flexibility. ROM is definitely one of the most frequently used terms to describe this physical capacity. While variables such as elasticity, compliance, passive compliance, passive stiffness, passive elastic stiffness, passive viscoelastic stiffness, passive resistance, extensibility, passive extensibility, passive moment and so on are terms aimed at describing what is commonly thought of as flexibility (Gajdosik, 2001), these concepts describe different tissues ability to deform or resist deformation, and should not be confused with ROM. ROM may be defined as the angles that a joint can actively or passively travel through. In this thesis ROM will be used to describe and refer to joint flexibility and maximal ROM will be used to describe the maximal capacity of ROM.
Further, *extensibility* is defined as the muscles-tendon unit's ability to lengthen to its fullest length. Further more, Gajdosik defines *passive extensibility* as the muscles ability to lengthen unaccompanied by muscle activation. Passive extensibility is the distance between initial muscle length and the maximal length and they are both determined by

the passive resistance to stretch. Passive extensibility affects the maximal length because the maximal length is the end point of the length of a muscle's extensibility (Gajdosik, 2001). ROM may be restricted by voluntary and reflex control, myogenic restraints such as passive and active resistive properties of the muscle, joint constraints such as the structure of the joint, bones, cartilage, ligaments, joint capsule or by soft tissues like skin and connective tissue. Individual perception and tolerance for pain may also limit maximal ROM (Alter, 2004).

3.2.1 Neural mechanisms: Implications for ROM

Although it is this topic is not the main focus of the present thesis, possible neural adaptations to stretching cannot be completely neglected. There are three main receptors may influence the effect of stretching exercise and preserving optimal ROM. These are the muscle spindles, Golgi tendon organs (GTO) and the mechanoreceptors of the articular joint. The muscle spindles are proprioceptors located in a capsule providing information about muscle length, the intrafusal fibers, or outside the capsule, the extrafusal fibers. The spindles are fasten at both ends to the extrafusal fibers and are oriented in parallel to these. Thus, when the muscle is elongated the spindle is stretched as well. GTO are mechanoreceptors innervated by Ib afferent neurons and are sensitive to muscle contraction. GTO are mainly located at the aponeuroses or the MTJ. GTO lie immediately in line with the force transmission for muscle to bone and are, in contrast to muscle spindles, thought to be in series with the muscle. When a muscle-tendon unit is stretched, the stretch subjected to the GTO straightens the collagen fibers and therefore compressing nerve endings and leads them fire. In summary, there are two noteworthy mechanoreceptors, the muscle spindles and the GTO.

There are two examples of modified reflexes in dancers compared with well-trained athletes. Nielsen et al (1993) demonstrated that the Hoffmann reflex and the disynaptic reflex were lower in ballet dancers. The authors speculate whether the prolonged co-contractions required by the classical ballet regimen may have led to tenacious decreases in the Ia-synapse and reciprocal inhibition. It has also been suggested that long-term stretching training may alter the composition of the tendon tissue and that these alterations may result on less loading on the muscle spindles, given equal tendon-tap force (Alter, 2004). Viidik (1973) showed that stretched tendons

display at tendency towards remaining in the stretched state. Therefore, any ensuing loading will result in less force being transmitted to the muscle.

In summary, the nervous system functions through a highly complex arrangement of reflex- interactions. Three central reflexes are the stretch reflex, reciprocal inhibition and the myotatic reflex. Further, antagonist co-activation/co-contraction has been observed during different movement tasks. Neuronal action may induce unyielding alterations in the central nervous system, which in turn may have implications for developing larger ROM.

3.3 Tendon properties

3.3.1 Tendon: Definition

Tendons merge with the muscles and aponeurosis of muscles at the myotendinous junction (MTJ). Distal to the MTJ is the part of the tendon that is often referred to as the *free tendon,* which then intermeshes with fibrocartilage at the osteotendinous junction (OTJ). The complex anatomy of the free Achilles tendon (free AT) illustrates the different tendon parts perfectly with its connection to the triceps surae group, consisting of Gastrocnemius Medialis (GM), Gastrocnemius lateralis (GL) and Soleus (SOL). GM and GL are proximally connected to the free Achilles tendon, and later SOL. Distal for the SOL myotendinous junction the tendon tissue proceeds as the free AT and then inserts on the calcaneus bone (Magnusson et al., 2003). Different properties between the aponeurosis tendon and the Free AT have been previously shown by research groups (Magnusson, Hansen, Aagaard, Brønd, Dyhre-Poulsen, Bojsen-Møller, & Kjaer, 2003; Finni, Hodgson, Lai, Edgerton, & Sinha, 2003). In this study the entirety of the triceps surae muscle-tendon complex have been investigated. Therefore, the term tendon refers for the length of the tendon from GM myotendinous junction to the calcaneal insertion, while the tendon distal for SOL myotendinous junction is referred to as the free AT. Please note that the role of the aponeurosis has not been taken into account in this particular project.

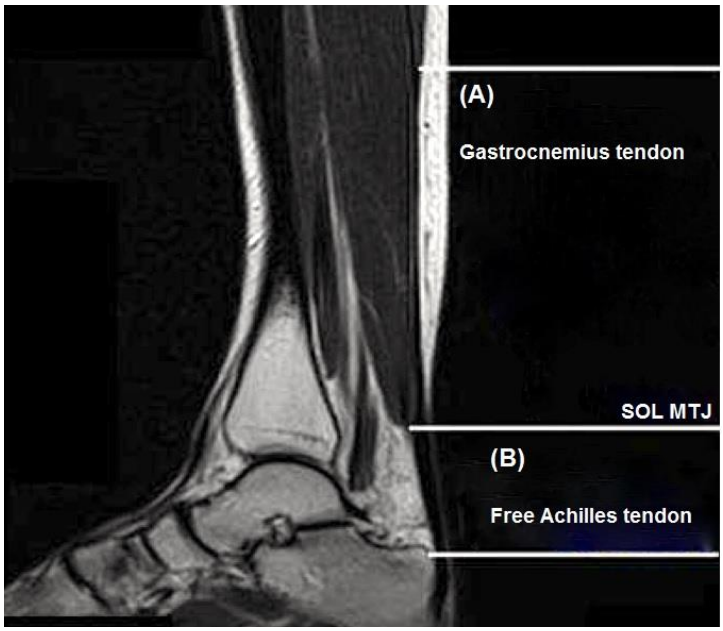


Figure 1: Definition of tendon components. Medial view. A: Gastrocnemius Medialis tendon (GM tendon). B: Distal part of Soleus myotendinous junction to free Achilles tendon (free AT) insertion on calcaneus. (Adapted with permission from Villars, 2013)

3.3.2 Tendon morphology and adaptation in morphological properties to different loading conditions

Tendon cross-sectional area (CSA) and tendon length are regularly used to quantify tendon morphological properties. Depending on type of tendon and mammalian, tendon dimensions are extremely varying (Ker et al., 1988). The morphological differences are most likely due to demand of different tendon functions and subjection to different mechanical loads (Ker et al., 1988; Roberts & Azizi, 2011; Lichtwark & Wilson, 2005b). Mechanical loading of tendons may influence the morphology of the tendon, seen as change in the dimensions of tendon CSA or tendon length. The human Free Achilles tendon CSA spans from 34mm^2 to 128mm^2 (Rosager et al., 2002; Kongsgaard, Kjaer, & Magnusson, 2005; Kongsgaard, Reitelseder, Pedersen, Holm, Aagaard, Kjaer, & Magnusson, 2007; Magnusson et al., 2001, Magnusson & Kjær, 2003; Hansen et al., 2005, 2011). Along the length of both the Free Achilles tendon and the patellar tendon regional CSA differences have been observed (Caroll et al., 2008; Kongsgaard et al., 2005).

Animal studies of tendon CSA in response to mechanical loading have been ambiguous, showing an increase (Sommer, 1987; Woo, Ritter, Amiel, Sanders, Gomez, Kuei, Akeson, 1980), decrease (Woo, Gomez, Woo & Akeson, 1982) or no change (Buchanan & Marsh, 2001) after loading. Until recent times, literature on the effect of physical activity on the human Free Achilles tendon has been incoherent. Kallinen & Suominen (1994) found larger patellar tendon with, but no difference in tendon CSA in older athletes compared to controls matched for age. Rosager et al. (2002) measured tendon CSA 3 cm proximal to the calcaneus insertion, and reported that habitual runners had 22% larger CSA than matched controls. On the other hand, Hansen et al. (2003) found no difference in CSA after a 9 months endurance training intervention in untrained human subjects. Magnusson & Kjaer (2003) extended the knowledge of these studies, reported region-specific tendon hypertrophy as a result of habitual loading in more experienced runners than in in the study by Hansen et al. (2003). Magnusson & Kjær (2003) showed that the CSA of the Free Achilles tendon varied along the whole length, with the most distal portion displaying 51-85% larger CSA than the most proximal portion. The most distal portion of the tendon in the runners had 36% larger CSA than the controls. This might be validated by studies reporting increased collagen synthesis after one bout running of 36 km (Langberg et al., 2009). It has also been theorized that muscle size may be related to the tendon CSA, and that the magnitude of loading, that is muscle strength, affects tendon size. Indeed, increased free Achilles tendon CSA and increased maximal plantarflexion moment was demonstrated after 14 weeks of induced high – or low cyclic strain (Arampatziz, Karamanidis & Albracht, 2007). Kongsgaard and co-workers reported 6-7% larger CSA at the proximal portion, accompanied with larger quadriceps CSA after 12 weeks of light or heavy strength training (Kongsgaard, Reitelseder, Pedersen, Holm, Aagaard, Kjær & Mangusson, 2007). Moreover, interesting findings in the study of Couppé and colleagues (2008), showed that intra subject differences in patellar tendon CSA was clearly related to loading conditions. Patellar tendons on both legs in elite fencers and badminton players were examined and compared to active controls. While the active controls showed no contralateral difference in knee extensor MVC, the athletes were on average 22% stronger in their lead extremity. Interestingly, the athletes had larger patellar tendon CSA (20-28%) in their lead extremities compared to the non-lead extremities Couppé et al., 2008). This was the case in the proximal and distal portion of the tendon, indicating a region specific tendon hypertrophy in response to the habitual loading on the preferred

extremity. The abovementioned studies are evidence that the magnitude of loading is an essential factor for tendon response and adaptation.

3.3.3 Tendon structural properties

Tendon tissue is characterized by its large content of extra cellular matrix (ECM) and rather few cells, which are mainly fibroblast-like Tenocytes. The tenocytes task is to produce ECM components like collagen, fibronectin and proteoglycans in order to repair tendon injuries and retain tendon homeostasis (Heinemeier & Kjaer, 2011; Wang et al. 2012). 55-70% of the tendon consists of water, where a large part of this is linked to the content of ECM. The ECM is composed of proteoglycan proteins, the specialized glycoproteins fibrillin, fibronectin, and tenascin C among other proteins represented in very small amounts. In addition, tendons contain collagen, ground substance and a small amount (<2%) of inorganic substance. Approximately 60-85% of tendon dry weight is collagen, and most of this is collagen type I (ca 60%). Of the collagen types type I represents 95%, however collagen type III (ca 5%) and traces of other types are also existent. Only 2% of the dry weight is represented by elastin, but this structural protein is of great importance for tendon function (Kjaer, 2004).

Tendon fibroblasts have a columnar arrangement seen along the force-transmitting axis of the tendon and transmit via gap junctions that connect to the ECM through integrin proteins (Heinemeier & Kjaer, 2011). When subjected to mechanical forces tendon cells respond by changing their gene expression, their protein synthesis and cell proliferation. These early adaptations may continue and instigate long-term structure alterations and lead to changes in tendon mechanical properties (Wang, 2006).

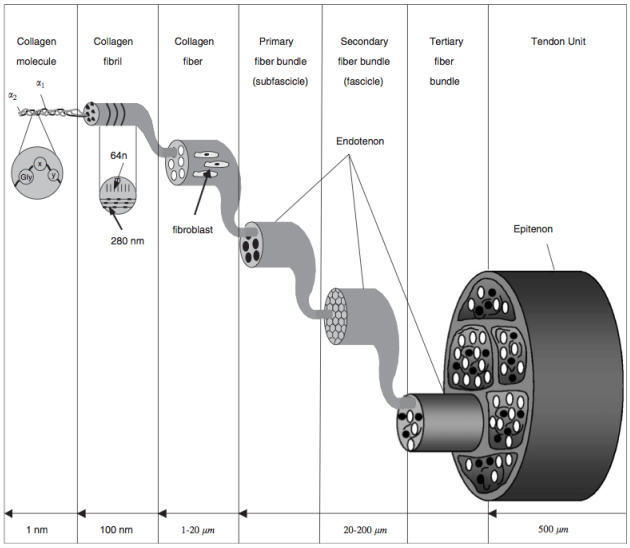


Figure 2: Multi-unit hierarchy of tendon composition. (Wang, 2006)

The tendons tissue is densely packed with highly organized collagen primary fibrils, fibers, fiber bundles and fascicles that together form the tendon unit. In comparison to muscles tendons have relatively less vascularization, namely ~1-2% of the ECM (Kjaer, 2004; Wang, 2006). Vessels spread from the epitenon where they run longitudinally into endotenon, a loose low-frictional matrix structure that facilitates gliding. The endotenon has both lymphatic and blood vessels, in addition to nerves and thus serves as metabolic supplier for tendons (Kastelic et al., 1978; Silver et al, 2003). Furthermore, some tendons, like the Achilles tendon, are surrounded by a connective tissue lined with synovial cells, paratenon. Paratenon also contributes to low friction with adjacent structures and allows for large movement of the tendon (Kjaer, 2004; Wang, 2006). The hierarchical composition of tendon lines fiber bundles along the axis and provides tensile strength. This micro-structural property is known as crimp. At rest tendon fascicles bend to a certain extent with a given crimp-angle. These crimp angles decrease in the first period of loading in advance of fiber elongation (Kastelic et al., 1978,Silver et al., 2003).

A collagen molecule is folded into a triple helix and several triple-helice polypeptide chains are cross-linked together. Therefore, collagen molecules may not move freely when mechanically loaded. (Wang, 2006 ; Heinemeier &Kjaer, 2011). Even though the collagen fibrils are mainly organized in the longitudinal plane in relation to muscle force acting on the tendon, however one may also find fibrils

organized in the horizontal and transverse plane. The hierarchic organization of this three dimensional structure is part of what gives the tendon its unique viscoelastic properties. Because tendons are organized in three planes, they are able to absorb longitudinal, transverse, horizontal and rotational forces during various types of activities.

Immediately at their enthesis (proximal: myotendinous junction, distal: osteotendinous junction) a different structure than the rest of the tendon is displayed. These differences are principally due to functional and mechanical demands. The tensile forces are four times higher at the osteotendinous junction that at the mid-tendon, thus the transition is strengthened with hyaline fibrocartilage, which distributes the forces (Wang, 2006; Benjamin, 2006).

3.3.4 Tendon mechanical properties and adaptation to different loading conditions

Mechanical properties of tendons are consequential for their function in the skeletal-muscle system (Matson et al., 2012). The mechanical properties will determine to what extent a tendon is able to stretch, shorten or potentially fail under loaded conditions. With insufficient tendon adaptation a load applied with higher force that what the tendon can bear, represents an increased risk of injury (Maffuli et al., 2005). Accordingly, mechanical properties of tendons are of extensive interest in sports sciences and medicine. There is high incidence and prevalence of injuries in the related to the lower extremities, and in particular the triceps surea complex and Free Achilles in weight bearing sports like running and dancing at a professional level (Hincapié , Morton & Cassidy, 2008; Nilson, Leanderson, Wykman & Strender, 2001). In fact the Free Achilles tendon has been suggested to be among the structures in the dancers body that are indeed susceptible to overuse injury (Maffulli & Denaro, 2012). For decades several studies that aim to investigate the material and mechanical properties of tendons have been completed. The conventional output variables of mechanical research testing may be: stress, strain, compliance, stiffness and Young's modulus (Table 1). The magnitudes of these properties vary considerable depending on tendon, specie, individual, and of course methods for measuring mechanical properties (Matson et al., 2012).

Table 1: Definition of important tendon mechanical properties

Tendon stress	*Dependent on tendon CSA and Force* *Ratio of load induced elongation to the unloaded length.* $\text{Stress} = \frac{F_t}{\text{CSA}} \left[\frac{N}{mm^2} = Megapascal\ (MPa)\right]$ *F_t = tendon force, CSA = tendon CSA*
Tendon strain	*Dependent on tendon CSA and original tendon length* *Load normalized to tendon CSA.* $\text{Strain} = \frac{\Delta L}{L_0}\ [\%]$ *ΔL= Length change, L_0 = original tendon length*
Tendon compliance	*Inverse parameter of tendon stiffness.* *Dependent on tendon CSA and original tendon length* $Compliance = \frac{1}{Stiffness} = \frac{\Delta L}{\Delta F_t} \left[\frac{mm}{N}\right]$ $\Delta F_t = Change\ in\ tendon\ force\ , \Delta L = Change\ in\ tendon\ lengh$
Tendon stiffness	*Inverse parameter of tendon compliance.* *Elongation in relation to applied tendon force* *Dependent on tendon CSA and original tendon length* $Stiffness = \frac{\Delta F_t}{\Delta L} \left[\frac{N}{mm}\right]$ $\Delta F_t = Change\ in\ tendon\ force\ ,$ $\Delta L = Change\ in\ tendon\ lengh$

Tendon mechanical properties have been investigated in isolated specimens of humans or animals. Under *in vivo* conditions, tendon length, maximal elongation and CSA are traditionally measured with ultrasound. One can theoretically assume that a

tendon that is longer and/or thinner will have lower stiffness than a shorter or thicker tendon given that tendon composition and structure is equal. However, if two tendons with different CSA and otherwise identical features were to be stretched to the same length, more force would be required to stretch the tendon with the largest CSA. In turn the tendon with the largest CSA will store more energy in comparison to the tendon with a smaller CSA. Methodology involving recording of force and elongation when stretching a tendon to failure has led to the characteristic curve-linear slope with 4 distinct regions in the tendon force-elongation plot (Figure 4).

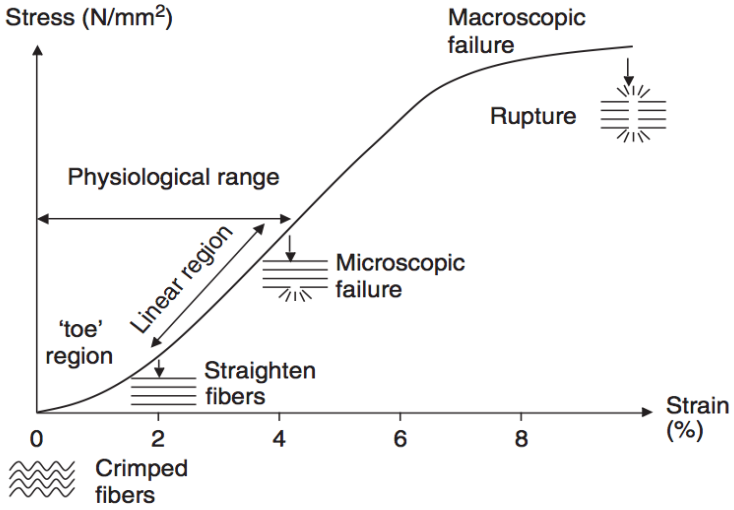


Figure 3: Classic stress-strain curve for tendons loaded to failure (Wang, 2006).

The stress – normalized strain curve represents tendon material properties. The first of 4 regions is the "toe region", which the first 2% of tendon elongation and is associated with reducing crimp-angle. Stress-strain values caused by normal physical activity happens in the toe region. In the "linear region" (2-4%) elastic fibers are in fact elongated. This makes the linear region interesting when investigating mechanical properties in vivo, and it is the region where tendon stiffness and modulus is calculated (Wang, 2006). When stretching a tendon over 4% micro-ruptures in the collagen fibers may occur. If stretched 8-10% macro–ruptures can occur and above this are the tendon can rupture completely. In late areas of the linear region, in micro-failure region and macro-failure region the change in tendon fiber length is plastic and they will not return to their original length post-loading (Wang, 2006; Silver et al., 2003; Magnaris &Paul, 1999, 2002). The abovementioned numbers are generalized values from *in vitro* studies

(for review please see Butler et al., 1978) and large variations in tendon properties have been measures *in vivo*. *Plasticity* is the crucial ability that enables tissue to change dimension permanently. Muscle fibers are plastic in the respect that they lengthen by adding sarcomeres, while tendons are considered somewhat plastic due to creep and lengthening of collagen fibers under sustained strain.

The stiffness of a tendon is expressed by change in force (ΔF) divided by change in deformation (ΔL), and is recognized as an objects ability to oppose length change. Thus, tendon length and CSA have implications for tendon stiffness.
Young's modulus is tendon stiffness normalized by tendon CSA and length. Thus, modulus describes tendon material properties and makes it possible to compare tendons with different dimensions. If the dimensions of two tendons are equal, the tendon with high Young's modulus is a rigid tendon, whereas the tendon with low Young's modulus values indicates a compliant tendon.

Table 2: Material properties of the tendons: Young' modulus (elastic modulus)

Young's modulus	*Relationship between tendon stress and strain. Independent of tendon CSA and tendon length. Allows to compare structures with different dimensions*

$$Young's\ modulus = \frac{Stress}{Strain}\ [GPa]$$

Tendons are known to hold viscoelastic properties (Taylor, Dalton, Seaber & Garret, 1990; Kjaer, 2004; Heinemeier & Kjaer, 2011). Viscous properties have been defined as an object that does not return to its original shape after having been subjected to stress. Elasticity, on the other hand, is a property that deals with Hooke's law (Hooke, 1678). Briefly, Hooke's law is a principal of physics that states that stress is directly proportional with strain, under a prerequisite that the given object does not change form permanently. Because tendons are viscoelastic they act neither perfectly elastic nor perfectly viscous due to loss of energy. The energy is most likely lost as heat and this phenomenon is known as, hysteresis. Hysteresis can be observed in the force-elongation relationship when a tendon is loaded and subsequently unloaded. The force-elongation curves will form a loop and the area within the loop (between the load- and

unload curves) depicts strain energy lost as heat. The tendon inhibits another time-dependent behavior, which is called *stress-relaxation*. When tendon tissue is held at a constant strain rate the stress in the tissue decreases. Conversely, when a loading force is constant, strain in the tissue will increase and relative tendon deformation will become less. This is referred to as *creep* (Heinemeier & Kjaer, 2011).

In conclusion, viscoelastic properties affect the tendon's capacities to convert muscle contraction into skeletal movements as well as joint stability (Duenwald et al., 2009; Matson et al., 2012; Roberts & Azizi, 2011).

3.3.5 Investigating tendon properties

To calculate the abovementioned properties initial tendon length, tendon deformation and CSA must be measured. The most common way of investigating these properties have been *in vitro* studies, where tendon biopsies have been utilized. *In vitro* studies have been highly useful and have for example shown that the tendon stiffness is far from uniform along the tendon path. However, one can assume that *in vitro* investigations are not mirroring the actual stress tendons are exposed to in different movement tasks.

Over the last few years the advancement of *in vivo* techniques for measuring both patellar- and Achilles tendon properties have progressed. Modern imagining techniques like magnetic resonance imaging (MRI) and ultrasound are now typically used to investigate tendon properties. Some tendon properties may be quantified and assessed in an isometric ergometer, where change in isometric torque and tendon deformation measured with ultrasound is synchronized (Ito et al., 1998; Fukunaga et al., 1996, Magnusson et al., 2001; Reeves et al., 2003; Seynnes et al., 2011; Kongsgaard et al., 2011). This method proved to be valid and reproducible (Hansen et al., 2006). Yet, one aims to record relatively small elongations, and one study (Schulze et al., 2012) on the patellar tendon has shown it is therefore necessary to do of multiple recordings to be able to produce reliable measures of tendon elongation. One can assume the same principle applies to recordings of the Achilles tendon. Further, calculated tendon stiffness is dependent on strain rate, where tendon stiffness will increase with higher strain rate (Theis et al., 2012). This precondition makes it challenging to compare

stiffness values across studies where different strain rates have been utilized. Theis and co-workers aimed to compare active stiffness with passive stiffness in the Achilles tendon, which led them to calculate stiffness in the toe-region of the stress-strain curve and resulted in relatively low stiffness values. Stiffness is not usually measured in this region, therefore one might speculate that their results reflects other properties that the actual stiffness of the Achilles tendon.

Methods have also been developed and used to measure tendon elongation during rest for further calculation of stiffness (Mizuno et al., 2011; Morse, Degens, Seynnes, Magnaris, & Jones, 2008). The methods of Morse et al. (2008) and Mizuno et al. (2011) have shown strong correlations with active (isometric) stiffness measurements, even though the average stiffness from the active method was higher than those from the passive method. Because of its independence of tendon dimension, differences in Young's modulus can point to changes in tendon material properties (Heinemeier & Kjaer, 2011). Some training studies have shown changes in Young's modulus without changes in tendon dimension (Arampatzis et al., 2010; Reeves et al., 2003) or increase in both (Seynnes et al., 2009).

3.3.6 Metabolic response in tendons to different types of loading conditions

It is well recognized that ECM turnover in tendons is responsive to physical activity. Blood flow, oxygen demand and rate of collagen synthesis increase with mechanical loading. Both animal and humane training studies have shown that physical activity leads to sustained increase in collagen turnover.

The tendons matrix cells are biosynthetic and metabolic. Tendons are thought to adapt their mechanical properties to function and demand, thus it is likely that tendons also will regulate their metabolic activity to changes in mechanical loading (Joza & Kannus, 1997). Mechanical forces are essential to morphological and functional muscle- and tendon cells and tissues. On a cellular level mechanical forces impact cytoskeletal arrangement, gene expression, proliferation and cell survival (Wang, 2006).

Tendon fibroblasts being the dominant cell type in tendons, seems to play a central role in the adaptations to mechanical loading and mechanotransduction. Strain of the tendon seems to induce mechano-sensitive responses in fibroblasts through integrins (Figure 4; Heinemeier & Kjaer, 2011; Wang, 2006). Integrins physically connect fibroblasts and ECM. The integrins are also positioned to convert mechanical forces into chemical signals and transmit these signals onwards (Katsumi et al., 2004). Further, fibroblast induced metabolic response mediates collagen synthesis and can lead to morphological changes with effects on tendon dimensions as well as mechanics.

In an *in vitro* study of human patellar tendon fibroblasts Yang et al. (2004) have found that when stretched in a cyclic manner the tendon fibroblasts increased their proliferation and the protein production of collagen type I and TGF-β1, which in part mediates collagen type I production. Maeda et al. (2009) exposed rat tail tendon fibroblasts *in vitro* to cyclic loading and found a dose-response relationship between strain magnitude or exposure time and fibroblast metabolic response. The higher strain magnitude or exposure time, the larger was the metabolic response.
The increases seen in fibroblast and collagen synthesis are in line with findings of humane *in vivo* studies (Hansen et al., 2008; Langberg et al., 2009). With a microdialysis technique in the peritendinous area of the Achilles tendon in runners before, acutely after and 72 hours after 36 km of running (Langberg et al., 2009), it was demonstrated that exercise induces changes in metabolic and inflammatory activity in the peritendinous region. In the 2 hours after running procollagen peptide concentration was decreased in comparison to baseline concentrations. However procollagen peptide in the tissue increased threefold compared to baseline after 72 hours. The research of Langberg et al. strongly indicates an upregulated collagen synthesis in response to acute loading.

The loading induced changes in tendon mechanical properties can be related to the increased collagen content subsequent loading. Further, altered collagen fibril density and size have been suggested to account for changes in mechanical properties after long-term loading. Collagen fibrils with larger cross sectional area should in theory increase tendon stiffness and Young's modulus, because larger fibril diameter gives better opportunities for cross-linking. Furthermore, only a few animal studies have been concerned with the effects of training on intratendinous organization, and the results

from these studies diverge. In some animal studies repeated mechanical loading leads to an increase in collagen cross-linking to secure reduced stress at failure. This in turn has implications for mechanical properties in general (Heinemeier & Kjaer, 2011). Couppé and co-workers have researched collagen content and cross-linking in old and young men (Couppé et al. 2009). Their findings show increased enzymatic and non-enzymatic cross-linking, but decreased collagen content in the older men compared to the young controls. There were no significant changes in mechanical or morphological properties. It might be that the increase in collagen cross-linking seen in the older men was a compensation for decreased collagen content. The results from Couppé et al. are supported by other authors (Kjaer, 2004; Heinemeier & Kjaer, 2011).

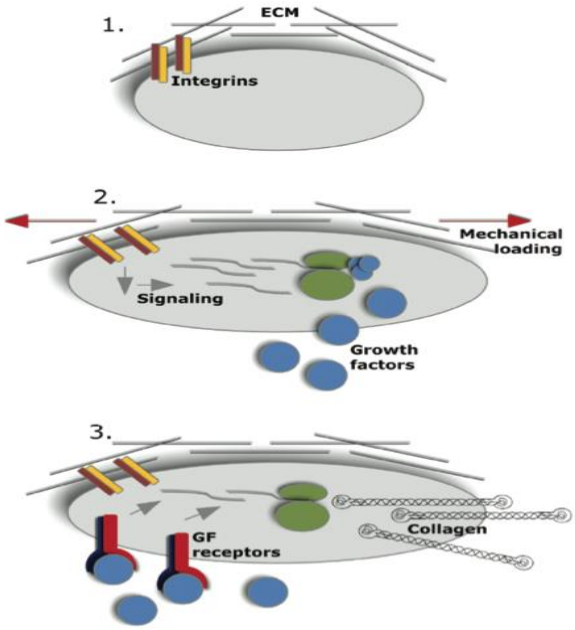


Figure 5: Possible mechanisms for tendon metabolic response to loading induced collagen synthesis. 1.) Fibroblast attaches to extra cellular matrix via integrins. 2.) Growth factors for transcription and are induced mechanical loading via intracellular signal transmitting. 3.)Autocrine and paracrine activity results in increased collagen transcription and synthesis. (With permission from Heinemeier & Kjaer, 2011)

Studies on humane tendon matrix have almost solely focused on collagen metabolism, however other components might also have a role in tendon adaptation to different loading conditions. Some studies have indicated an involvement of hydrophil molecules, such as glycosamines and proteoglycans. Further, humane studies have reported decrease in crimp-angle and reduced water content with aging or disuse (& Magnaris,2006; Kjaer, 2004).

3.4 Biomechanical, morphological and structural properties of skeletal muscle

3.4.1 Biomechanical properties of muscle

Viscous and elastic mechanical properties of muscles resist deformation and therefore have implications for ROM. We are here concerned with 1.) Parallell elastic (PEC) components, 2.) Series elastic elements (SEE) and 3.) Contractile components (CC) The PEC consists of sarcolemma, sarcoplasma and elastic fibers and lies parallel to the contractile machinery. PEC is responsible for passive muscle tension. Tension is developed when a muscle stretch is initiated, and as the stretch increases more tension is developed. Titin may be the source of this tension and several studies have demonstrated that upon destroying titin, muscle tension decreases (Horowitz, Kempner, Bisher & Podolsky, 1986; Yoshioka, 1986; Funatsu, Higuchi & Ishiwata, 1990). reported that tension decreased with degradation of titin. During stretching actin and myosin did not change length. However, the filaments slid past each other (sliding filament theory). When stretching is initialized the sarcomere resists deforming with moderate tension, which after substantial stretch will increase sharply and oppose further stretching. When stretching is released titin reacts, thus titin is able to store elastic energy. The functional role of the SEE in the MTU is further elaborated in chapter 3.9.1. The CC are represented by the actin and myosin filament cross-bridges, and it is the number of cross-bridges in the physiological CSA (CSA) that determine the muscles maximal force in an isometric muscle action. The higher concentration of CC in a given CSA, the higher the maximal force (Raastad, Paulsen, Refsnes, Rønnestad, & Wiesnes, 2010).

3.4.2 Muscle morphology and adaptation in morphological properties to different loading conditions

Like the tendon, skeletal muscle tissue holds adaptive properties that will change the dimension of the muscle itself and of its components, i.e. hypertrophy and atrophy. Stimuli causing muscle hypertrophy may be mechanical, metabolic stress and the body’s hormonal balance. In strength training mechanical stimuli affects mechano-

sensitive proteins, which then start a signalling, cascade relaying on the signals leading to muscle growth. Studies have also shown that inducing a metabolic stress on the muscles that transiently reduces blood flow in the working muscle may compensate for a sub-optimal mechanical stimulus. It seems that the local stimuli, i.e. the mechanical stimuli and metabolic stress, are the two main contributors to hypertrophy and they both involve activation of local growth factors (IGF-1, mechano growth factor, hepatocyte growth factor and fibroblast growth factor), down regulation of myostatin and initiating of intracellular signalling pathways regulating the transcription of genes involved in hypertrophy. The signalling pathways for hypertrophy involve both muscle fibres and satellite cells in parallel. In this way activated satellite cells will contribute with more cell nuclei to the muscle fibers that are stimulated to increase in volume. Thus, the muscle protein synthesis increase in line with muscle fiber growth.
Even though the acute hormonal changes occurring post strength training does not seem to play any role of significance in muscle growth, changes in the baseline levels of testosterone, cortisone, growth hormone and insulin might affect the body's total muscle mass (Raastad et al., 2010).

In the hypertrophy response the muscle increase mass and CSA. The reasons for these increases are that the amount of contractile protein content (actin and myosin). Further, the increased protein content results from increase in myofibril size and number (MacDougall, 1992). The myofibrillar adaptations lead to increased maximal strength due to more contractile components in parallel. The muscle cell volume is also a factor affecting muscle dimension. The volume of sarcoplasmatic reticulum (SR) and the amount of SR related proteins grow in accordance with the increased muscle fiber CSA, to keep the SR volume constant (Raastad et al., 2010).
Briefly, atrophy is the inverse effect of hypertrophy. Disuse or immobilization may induce muscle atrophy, where the muscle fibers will decrease in diameter and the number of fibers decreases (Raastad et al., 2010).

Finally, skeletal muscle may not only increase in CSA, but also in length. During the course of growth from an infant to adult our muscles are continuously being stretched due to skeletal development. The mechanism for adapting to growing bones seems to be adding more sarcomeres in series in each myofibril. To our knowledge, in fully-grown adults neither muscle origin, insertion nor bone length may change in

length due to mechanical stimuli or metabolic stress, consequently muscles adapt by increasing, which be elaborated in the following chapter.

3.4.3 Muscle architecture

The terminology *muscle architecture* is used to describe muscle fiber organization, hereunder muscle fascicle length, pennation angle and muscle thickness. Muscle fascicle organization can be altered with repeated mechanical stimuli, such as strength training, stretching exercise (Tabary et al., 1972; Lima, Carneiro, de S Alves, Peixinho & Oliveira, 2014) (Aagaard, Andersen, Dyhre-Poulsen, Leffers, Wagner, Magnusson, Halkjær-Kristensen & Simonsen, 2001;) or because of pathological conditions (Zhao et al., 2011). Ultrasound technology allows for *in vivo* investigation of muscle architecture during rest, passive motions and active tasks. When it comes to studies assessing human muscle architecture in relation to stretching they are limited in number (Nakamura, Ikezoe, Takeno & Ichihashi, 2011; Abellaneda, Guissard & Duchateau, 2008; Morse et al., 2008; Samukawa et al., 2011,). Further, most studies have only assessed the *acute* effects of stretching on muscle properties.

Muscles that are chronically exposed to elongation seem to add sarcomeres in series in order to increase fascicle length. While this has been methodically challenging to study in human muscle, these adaptations have been well documented in animal studies (Tabary, Tabary, Tardieu, Tardieu, & Goldspink, 1972; Williams & Goldspink, 1978). E.g soleus in generations of mice was immobilized in a lengthened position. The newborn mice had ca 500 sarcomeres in series and after 6 weeks over 2000 sarcomeres in series were found (Williams & Goldspink, 1978). Muscle fascicle length has been assessed in a few human studies. Ultrasound imagining has been used for direct or indirect (fascicle length = muscle thickness•sinθ) measurements of fascicle length. After 13 weeks of strength training of knee extensors with dynamic squats to 90 degrees, an increased fascicle length in vastus lateralis of 10% was observed (Alegre, Jiménez, Gonzalo-Orden, Martín-Acero, & Aguado, 2006). Cross-sectional studies have also provided indications that muscle fascicles are longer in individuals with years of strength training (Breuche & Abe, 2002; Abe, Kumagai & Breuche, 2000; Kumagai, Abe, Breuche, Ryushi, Takano & Mizuno, 2000) This may result from hypertrophy since sumo wrestlers, sprinters and weight lifters had longer muscle fascicles compared

with controls matched for anthropometry (Raastad et al., 2010). Longer muscle fascicles display higher shortening velocities and higher mechanical power compared to shorter fascicles. Longer fascicles have a broader length range of force (force-length relationship) compared to shorter fascicles, which in turn may affect muscle performance (Stafilidis & Arampatzis, 2007). From a functional point of view the purpose of adapting muscle fascicle lengths might be optimizing the muscle properties to fit the conditions it is most often exposed to. The relationship between muscle lengths and force-production is well accepted. Actomyosin cross-bridges are uniformly distributed along the thick filament. When a muscle is elongated overlap the cross-bridges decrease. Therefore, the more the muscle is lengthened beyond the optimum range for force production, the lower the force is produced. To the authors knowledge it is not known whether years of habitual stretching exercise of human muscle influences muscle thickness or induce longer muscle fascicles.

The pennation angle of muscle fibers is one other factor that may affect the force magnitude applied to a limb. Seynnes and colleagues (2007) found increases in muscle fascicle lengths and pennation angles in human vastus lateralis after 35 days of high-intensity resistance training. Further, muscle hypertrophy was linked with the increases in both fascicle length and pennation angle. These responses suggest adding of sarcomeres both in series and in parallel of the muscle (Seynnes, de Boer & Narici, 2007). Strength gains in response to hypertrophy have been associated with increase in muscle fiber pennation angle (Seynnes et al., 2007) and maximal muscle force is presumed to increase with increasing pennation angles to the upper limit of 45 degrees (Aagaard et al., 2001). As a consequence, geometrically speaking pennate muscles are expected generate greater contractile force compared to non-pennate muscles. It is clear that muscle architecture is an important morphological factor for muscle function, illustrated by the length-tension relationship and further the force-velocity relationship. From a mechanical point of view the morphological (muscle thickness, muscle CSA, tendon CSA and tendon length), mechanical (length-force relationship of the MTU, stiffness, muscle force) and structural (pennation angle, fascicle length) properties related to force production affect the MTU's work-producing abilities (Biewener & Roberts, 2000).

3.5 The anatomy and function of the triceps surae and Free Achilles tendon

The triceps surae consist of soleus, gastrocnemius lateralis and gastrocnemius medialis. The muscle group is attached to the calcaneus trough the Achilles tendon (AT). This muscle-tendon complex mainly plantar flexes the talocruralis joint. The AT is the tendon with the greatest CSA in the human body and it acts as a classic energy storing spring which stores and returns a considerable portion of the energy required for example for movement tasks like hopping, of which dancers in particular practice extensively. Lichtwark & Wilson (2005b) combined motion analysis and ultrasonography to determine whole AT length changes during dynamic movement tasks. They managed to demonstrate the substantial energy storing- and releasing capacities of the AT. The tendon stretches proportionally to the force applied and then recoils to release a great amount of energy. In a high strain movement task like hopping 74% of the energy stored in the downward movement of the hop was released in the upward movement (Lichtwark & Wilson, 2005b). This means that the Achilles tendon provides a good 16% of the total mechanical energy of the hop. By many researchers and practitioners this is thought to aid as an energy saving mechanism in walking, running, jumping, leaping and bounding movements (Bobbert et al., 1996).

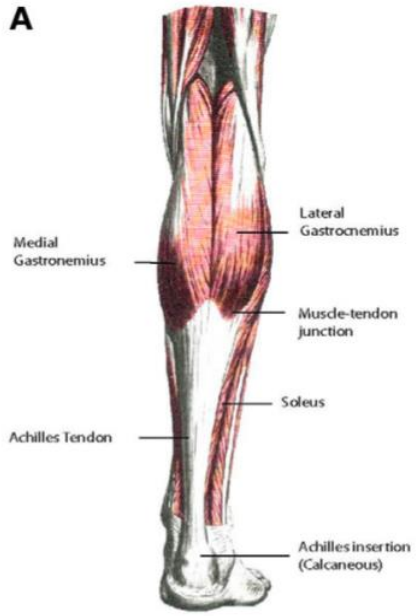


Figure 5: Anatomy of the triceps surae muscle group and the Achilles tendon (Lichtwark &Wilson, 2005b).

A large part of the MTU length change take place in the AT, and consequently the return of the elastic energy during tendon recoil supplies mots of the work essential for take-off. Juxaposed to this, the muscle and series elastic structures like the proximal tendon and aponeurosis, only stretch and shorten a little during the hop, hence the reduction in work required by the muscle fibres. This is viewed as energetically favourable and efficient due to less work required form the muscle fibres and reduced production of heat from actively contracting muscle fibres (Lichtwark & Wilson., 2005a). The aponeurosis of Gastrocnemius Medialis has also been found to be very compliant, therefore a proportion of the stretch and recoil taking place in the muscle fibres and aponeurosis might also be elastic strain energy.

3.6 Stretching exercise: Methodological clarifications

Clinicians and athletes aiming to increase ROM use several different stretching methods. The most commonly known methods are static stretching, dynamic stretching, ballistic stretching and proprioceptive neuromuscular facilitation (PNF). Professional dancers utilize all the methods stated above and in this chapter a very brief overview of these different methods would be given.

Static stretching and static progressive stretching: Taking slowly the MTU to the ROM of tolerance and holding it there for seconds to several minutes. In static progressive stretching the person will reposition the limb when a degree of relaxation is felt and a new end ROM will be held for an additional duration of time. Dancers most commonly use this method.

Dynamic stretching (not to be confused with ballistic stretching): uses controlled, gentle swinging movements to reach end ROM. Unlike static stretching the end position is not held. Dynamic stretching is commonly used as part of a sport-specific warm-up.

Ballistic stretching: Uses momentum to impose repeated high-speed and high-intensity bouncing movements on the MTU in attempt to force a limb beyond its normal ROM.

PNF: Involves a brief isometric contraction followed by a stretch, and is thought to increase ROM by reciprocal inhibition. (Alter, 2004)

3.7 Possible effects of stretching

3.7.1 Evidence for MTU adaptation in animal models

The most extreme effects of stretching on MTU morphological and mechanical properties can be found in animal studies and studies of isolated muscle or tendon. Acute *In vitro* studies on rat-tail tendon have showed consistent plastic lengthening (Warren et al., 1976; Sapega et al., 1981). Warren et al. (1976) demonstrated that the greatest residual tendon elongation was observed after low-load (6-10% of rupture load), long duration (50 minutes) static stretching, in comparison to vigorous (26-41% of rupture load), short duration (5 minutes). Taken together the data from the rattail studies state that 1.) The higher load magnitude, the higher rate was tendon elongation 2.) The proportion of tendon lengthening that is plastic is larger with combined low-load, long duration stretching (Sapega et al., 1981). Stolov and colleagues (1970) found that static stretching also lengthens the muscle. By stretching rat gastrocnemius for weeks with progressive tension for up to 5 minutes, they found 15-20% increase in muscle length (Stolov et al., 1970). Likewise, 4 weeks of immobilization in lengthened positions has showed an increase in muscle length in cat Soleus (Tabary et al., 1972). Inversely, 4 weeks of immobilization with the muscle in shortened position resulted in a solid decrease in muscle length in cat gastrocnemius. The changes in muscle length is thought to come from adding of sarcomeres in series in the lengthened position and a loss of sarcomeres in the shortened position, as an adaptation to the new functional length (Tabary et al., 1972). The increase in sarcomeres in series is modest (19%) compared to the robust decrease (40%) in sarcomeres when the muscle is shortened. Further, the effects of sarcomere loss and adding is only transient. When the immobilization ceased the muscles returned to its anterior state within 4 weeks (Tabary et al., 1972).

In vivo animal studies of peripheral denervation of skeletal muscle have demonstrated the evident loss of the ability to produce voluntary active tension. Post denervation demonstrated decreased extensibility and steeper passive tension curves compared with controls. Further, denervation studies have shown that adaptations in muscle length may result from a myogenic-, not neurologic mechanism. In another study by Stolov and co-workers (1971) the denervated muscles in adult rats were

immobilized in shortened position for 8 weeks and as a result displayed shortened muscle belly. Supporting evidence was reported from a study of adult cats, which after 4 weeks lost up to 35% of the sarcomeres (Stolov, Riddell & Shrier, 1971). The phenomenons observed from denervated muscle in shortened positions were the same as those from innervated muscle immobilized in shortened positions. Despite the fact that the regulation of sarcomere numbers may be independent of muscle activation level, both increased and decreased muscle activation seems to affect the regulation rate (Gajdosik, 2001).

Summarizing, it seems that both the animal muscle- and tendon tissue is responsive to static stretching of different durations and loads. When immobilized in shortened or lengthened positions, animal muscles responded by adapting their length to a new functional, operating range by adding or eliminating sarcomeres in series. Collagenous tissue in rats also responded to static stretch of different load and durations by lengthening plastically. Animal studies on peripheral denervation have provided evidence for the fact that length adaptations seen in immobilized muscle may be due to a myogenic mechanism, rather than a neurogenic mechanism (Gajdosik, 2001).

3.8 Human MTU Adaptations to Stretching Exercise

3.8.1 Acute Effects of Stretching Exercise

Alterations in the human muscle morphology, structure and mechanical properties after stretching training have yet to be verified. It has been advocated by clinicians and coaches that the application of heat and use of warm-up enhances the efficacy of stretching and increase maximal ROM. However, it has been theorized that the acute increase in ROM seen after the short-duration (15s-1min) stretches utilized in warm-up regimes indeed come from an increased tolerance to the stretch, rather than adaptations in the tendon (Magnusson, Aagaard, Larsson, & Kjaer, 2000).

While maintaining the holding phase or static phase for a static stretch, the stress will gradually decrease while the length will be upheld (Magnusson, Simonsen, Aagaard, & Kjaer, 1996a). Because stored viscoelastic energy is lost instantly after the MTUs are stretched they show viscoelastic stress relaxation, which may be expressed as

the percentage decrease in passive resistance over time. However, the process of stress relaxation seems to be reversible and does not contribute to long-term effects of stretching training. Many studies have showed an increased muscle length and maximal passive torque at the same angle following a single bout of stretching training (Magnusson et al., 1996a; Gajdosik, 2001), which has been associated with reduction of creep angle without changes in EMG activity. Because of this, a simultaneous increase in muscle length and maximal passive torque has been attributed to instant increases in tolerance to stretch, without change in the muscles' passive viscoelastic properties. Increased muscle length and maximal passive torque might be a consequence of muscle lengthening (strain) in relation to increased lengthening creep tension (stress). The acute effects of stretching seen as an increase in ROM may be due to an extended creep response. Creep can assist in explaining the immediate increases in maximal passive torque that have been measured in response to therapeutic stretching procedures (Gajdosik, 2001).

Rapid decrease of viscoelastic properties in the MTU has been seen in human Gastrocnemius after short-term (5 days) stretching exercise, with a static stretching volume of 5 times 1 minute (Mizuno et al., 2013). Acutely after stretching stiffness was decreased, however stiffness recovered within 15 minutes. Furthermore, Mizuno et al. (2013) found an increase in max ROM was observed as a result of the 5-day stretching exercise, but it seemed the decrease of viscoelastic properties was faster (>15 min) than the decrease of the new max ROM (>30 min) after stretching intervention. A stretching intervention of this duration and frequency did not produce chronic alterations in any parameter, but showed transient changes in mechanical – and viscoelastic properties. However the results might indicate that the time course for adaptations in mechanical properties and increased ROM are different. Arguably, it might take longer for mechanical adaptations to be detectable, than for maximal ROM to increase.

In summary, given that there are no anatomical constraints, there seems to be two possible causes for the limitation of MTU ROM. The mechanical properties essentially relates to the tissue stiffness. This parameter may be influenced acutely, owing the fact that these tissues are not perfectly elastic but also have a portion of viscosity (stress-relaxation/creep). The individual tolerance to stretch is the other limitation. This parameter will determine how near maximal strain one can stretch the

MTU of an individual before plastic changes occur. This suggests that if two individuals have identical MTU morphological and mechanical properties, their tolerance to stretch will determine the extent of their flexibility.

3.8.2 Long-Term Effects of Stretching Exercise on the MTU

In the literature use of the expression *long-term stretching exercise* vary greatly from 3 to 10 weeks (Weppler & Magnusson, 2010), and within each of these studies the training volume is evidently very different. The training volume and the time course may well be important factors in stretching exercise studies when aiming to detect changes in structural and mechanical properties in MTUs. In a recent study Lima et al. (2014) assessed the effects of a static stretching program on muscle architecture. The subjects stretched biceps femoris and vastus lateralis 3 times per week with 3 sets of 30 seconds. ROM increased, but no change in maximal knee flexion angle, isometric flexion- and extension torque or muscle architecture was observed. With the relatively low exercise volume (and possibly intensity) in the protocol it is not surprising that increased ROM was the only changed parameter. Further, this underlines the importance of training volume in such studies.

Many studies with various time courses have been conducted in the quest to uncover the true mechanisms behind increased ROM after stretching exercise. The results however remain divergent. There are studies that indicate increased ROM, decrease in MTU stiffness and altered viscosity after a stretching intervention (Kubo et al., 2002b; Nakamura et al., 2012; Gajdosik, 2001). Kubo et al, (2002b) found decreased passive torque and no change in Achilles tendon stiffness after 3 weeks of static stretching. However, hysteresis decreased by 12%. This indicates that the stretching changed the viscous properties, but not the elastic properties of the tendon. Even with a higher training volume and frequency (2 x 60 seconds daily, for 4 weeks) the results were similar with regard to passive torque and ROM, and no change in muscle architecture was found (Nakamura et al., 2012).

On the other hand studies have observed increases in ROM without the accompanying change in stiffness of the MTU (Magnusson, Simonsen, Aagaard, Sorensen, & Kjaer, 1996b; Ben & Harvey, 2010). It has been proposed that the increase

in ROM is not because of change in muscle- or tendon length, but is owed to a neural adaptation ie. increased tolerance to stretch. This theory states that both acute and long-term (3-8 wks.) stretching exercise leads to an alteration in the subject's tolerance to the sensation of stretching (Halbertsma et al., 1994; Halbertsma et al., 1996; Magnusson et al., 1996b). With lack of change in other parameters than ROM and torque angle, the sensory theory remains the only explanation for the increase in maximal ROM. However, possibly important properties (joint capsule, aponeurosis, muscle mechanical properties) remain uninvestigated or are not measured in the most optimal way (2D muscle architecture, length of MTU).

According to a review by Weppler & Magnusson (2010), it appears that while several long-term (>8wks) stretching training interventions have contradictory results, the difference simply lies in the definition of muscle extensibility. Studies that report increased muscle extensibility utilized a sensory endpoint that may demonstrate that the chosen sensation had its onset later during applied stretch, thus allowing increases in in muscle extensibility. Studies that reported no significant increase in muscle extensibility used an endpoint of standardized torque, which provide some support to the concept that there was no shift of the torque/angle curves or alterations in muscle stiffness (For full review, see Weppler & Magnusson, 2010). However, by solely relying on a sensory endpoint standardization of measurements will prove unworkable.

As previously addressed animal muscles have been immobilized in lengthened positions and subjected to high volume and high intensity stretching exercise. Whereupon new sarcomeres are in series as an adaptation to a new functional length (Tabary et al., 1972; Williams & Goldspink, 1978), and maximal ROM was increased. This mechanism may also apply in in human muscles, but the author's knowledge only one case study regarding a femoral distraction to correct for asymmetrical legs reported this (Lieber, 2010).

Adaptations in muscle length because of changed neurological activity such as increased tolerance to pain and antagonist contraction activity have supported the idea that passive length extensibility adaptations in human muscle could be independent of the level of neurological activation. Increased strength and function of antagonist muscle following surgical lengthening of the agonist muscle group supports the

hypothesis that functional alterations stems directly from alterations in muscle length, not from neurological changes (Gajdosik, 2001).

In summary, besides the typical increase in flexibility, almost nothing concrete is known about the mechanisms of adaptations to long-term (>8 weeks) stretching training. It is therefore interesting to investigate the potential differences in muscle architecture, tendon morphology and mechanical properties of the MTU between a group with a history of high stretching volume and intensity, and a group with little or no experience with stretching.

3.9 Functional consequences of long term stretching exercise in the MTU

3.9.1 The role of series elastic elements

During stretch-shortening actions some of the elastic energy can be stored and returned in the myosin cross-bridges, but the majority of the elastic strain energy is stored and returned within the aponeuroses and tendons of a muscle. The series elastic elements (SEE) lie directly in line with the contractile components of the muscle, and SEE consists of both active and passive elements.

While the active elements are the intracellular cross-bridges of contractile muscle proteins, tendons represent passive elastic elements that transfer force in series with active force generating fibres the a muscle (Biewener & Roberts, 2000). Thus, for tendons it possible to transmit forces relative to the amount of stretch they are subjected to. This mechanical property enables the tendons to store and return energy during locomotion and other movements. The compliance of the tendon is particularly important when determining a time course of muscle power output (Lichtwark & Wilson, 2005). The series elasticity in different MTUs (MTU) (muscle, aponeurosis and tendon) may differ depending on its role. Series elasticity has been recognized as an asset to the antigravity muscles of running animals owing to the tendon elongation that occurs during loading and the storing of elastic energy, which is returned later in the movement.

In muscles connected to a long external tendon, the series elastic stretch of the tendon may represent a significant fraction of its functional length range. Notwithstanding the fact that increased tendon length advantages greater elastic recovery, it will restrict the muscle's ability to control length changes.

4.0 Design and methods

4.1 Study design

This study was conducted in a cross-sectional design. The following paragraphs aim to provide the reader with an overview of the protocols for the study.

All testing was performed on the left leg. All the subjects underwent familiarization identical to the test procedures at least 24 hours prior to data collection, and the chronological order of the procedures is illustrated in the figure below.

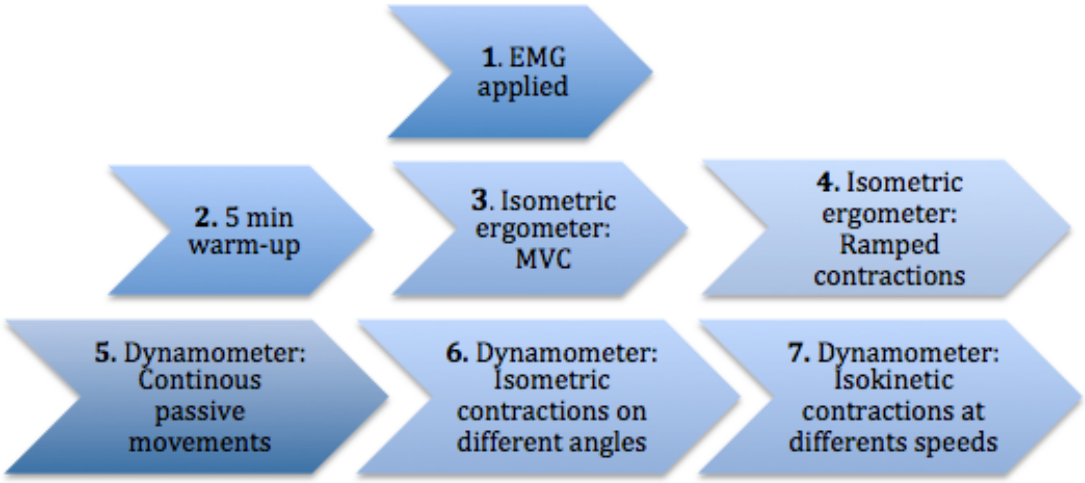


Figure 6: General test procedures. The procedures were identical on familiarization and test-day.

4.2 Ethical considerations

The subjects were informed that their participation in this research study was entirely voluntary and that they could withdraw their participation without stating any reason for this and without further consequences. All subjects who chose to participate signed a Certificate of Informed Consent (Appendix 2). The testing concerning the control group is part of an ongoing PhD project, for which the Ethical committee of Oslo, Norway granted ethical approval. As an amendment ethical approval for the present project was also applied for and granted (Appendix 1).

4.3 Recruitment and subject inclusion/exclusion criteria

14 female elite ballet dancers (age 31 ± 2.80, height 1.67 ± 0.06 m, weight 60 ± 0.06 kg) from the Norwegian National Ballet and freelance elite ballet dancers with similar training background (23.9 ± 5.08 years of practice, 21.4 ± 11.20 hours/week) were asked to participate in this study. All dancers agreed to participate. However, 4 dancers dropped out before or post familiarization, and reported this was due to overuse injuries related to the foot and/or ankle. Thereby, 10 dancers continued on to the main testing. 10 students (age 26.3 ± 4.19, height 1.66 ± 0.07 m, weight 59 ± 0.08 kg) from the Institute of Physiotherapy at Oslo and Akershus University College of Applied Sciences were matched with the ballet dancers by age height and anthropometric measurements, and served as control subjects. The subjects received both oral and written information about this study, and were further asked to confirm if they wished to participate in the study. The subjects filled out a questionnaire regarding their training background, stretching training back ground, dominant leg etc. (Appendix nr 4).

Fulfillments of the following criteria was determined on the day of familiarization:

Inclusion criteria for ballet dancers:

- Being between 18 years and 39 years
- No muscle- or skeletal diseases
- No ankle- or calf injuries the last 6 months.
- Regular stretching of the plantarflexor muscles for the last 10 years (more than 10 minutes of stretching, 3 times per week).

The control subjects had to fulfill the following criteria:

- Have turned 18 years, but not 39 years
- Have no muscle- or skeletal diseases
- Have no ankle- or calf injuries the last 6 months.
- Have not been stretching regularly the last 10 years (more than 10 minutes of stretching, 3 times per week).

4. 4 Procedures

4.4.1 Familiarization

The subjects then became familiarized with the all the equipment, the entirety of the test protocol, and performed the tests in the same sequence and with the same number of trials and repetitions as they would on the main test.

Test protocol overview

- US image collecting at rest, subject lying prone and sitting
- Warm-up
- Ramped contractions in isometric ergometer
- Maximum ROM in isokinetic dynamometer
- Passive torque up to max ROM in isokinetic dynamometer

4.4.2 Muscle thickness

US Images of the muscle thickness were taken from GM and SOL muscle bellies
The subjects were lying prone on a bench with their ankles hanging loosely on the outside of the bench to ensure neutral ankle joint position. Ankle joint angle was measured with a manual goniometer in this position. 2 images for each SOL and GM muscle thickness were obtained transversally, with the use of skin markers to ensure that both images were taken on the same area.

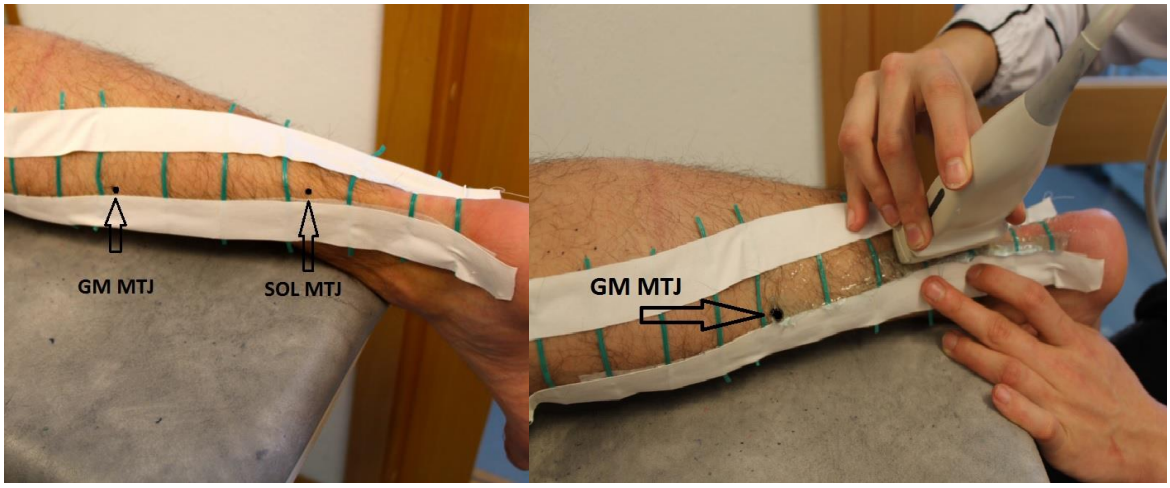
4.4.3 Muscle architecture at rest

With the subject still lying in the abovementioned position two US images of the GM and SOL were obtained. External skin markers were used to ensure that both images were taken on the same area.

4.4.4 Tendon length

The MTJs of SOL and GM were identified with ultrasound and the location was marked on the skin surface. A scaffold of adhesive strips and metallic wires was placed along the trajectory of the AT, based on the calcaneus insertion, SOL MTJ and GM MTJ. The wires were made of echogenic material casting dark vertical lines within the US field of view and thus served as a reference for image stitching and further analyses. Ultrasound

images were collected along the full length of the AT, with the first image taken at the calcaneal insertion and the last at the location of the GM MTJ.



A **B**

Figure 7: A: Adhesive scaffold with echogenic markers. B: Obtaining of tendon length US images from the calcaneal insertion to GM MTJ.

With the adhesive scaffold still attached to the leg, the subjects were seated in the isometric ergometer with their ankle joint at 0º and the hip joints in 90º flexion. The procedure of collecting images along the whole AT was repeated in identical manner. The back angle, hip angle and seat height were the same for all subjects. 3 US images were taken of the thickness at the proximal, distal and mid portion of the free AT.

4.4.5 Mechanical properties (Stiffness (F/δ))

To calculate agonist knee extension torque, antagonist co-contraction was estimated from EMG. The subjects the completed a 5-minute warm-up on a stationary bike (Monarch, 828E, Varburg, Sweden) at a self-chosen pace. After the warm-up a goniometer was attached to the foot and the subject was seated in the isometric ergometer (Figure 8). The test foot was placed on a plate with the ankle at 0 degrees dorsiflexion and the knee extended horizontally. A load cell was fitted to the footplate to measure plantarflexion and dorsiflexion force (Volt converted to Nm). The distance of the chair was set so that the subjects could fully extend their knee whilst heel movement was prevented. The foot was securely strapped to the footplate.
The malleolus of the ankle joint was aligned with the center of rotation of the isometric ergometer by inserting wooden planks between the footplate and the foot. During

testing the subjects instructed to keep their arms crossed over their chest and to limit their movements to ankle plantarflexion or dorsiflexion as appropriate. Goniometer and EMG data were recorded during all active and passive trials, and will be thoroughly described in later chapters.

To determine maximum force levels and to assess the size of antagonist co-activation during the isometric ramped contractions, the subjects performed 2 maximal voluntary contractions (MVC) in plantar flexion and 2 MVCs in dorsiflexion. There was 60 second (s) break between each of the MVCs. Variation in force of less than 10% was accepted. If the subjects did not achieve this within 2 trials, an additional trial was added. (Magnaris et al., 1998; Bojsen-Møller et al., 2004). For each trial an MVC was sustained for 5 seconds.

The displacement of the SOL MTJ and GM MTJ was scanned with ultrasonography during isometric ramp contractions. An ultrasound probe was secured to the leg, sagittal to the tendon (Figure: 8).

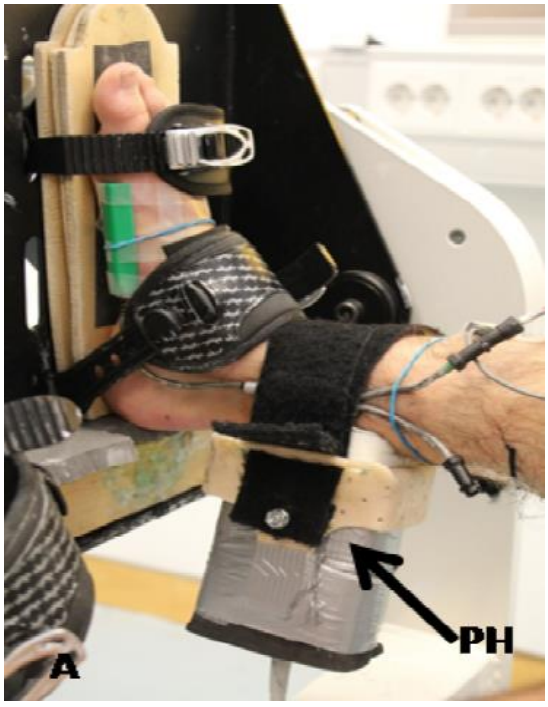


Figure 8: Probe holder (PH) secured to the leg with the probe here scanning SOL MTJ in the sagittal plane.

US video of the calcaneal AT insertion during 3 ramped contractions were also collected for correction of artefactual calcaneus rotation. For calculation of tendon stiffness the subjects performed ramped isometric contractions (IRC) up to maximum force. Prior to each ramp contraction the subject performed 3 small, sequential plantarflexions to condition the tendon (Magnaris, 2003), then rested for 5 seconds and then performed the IRC in plantar flexion. The subjects had a visual display with feed back on their performance, to ensure that all subjects would contract their muscles at the same loading rate.

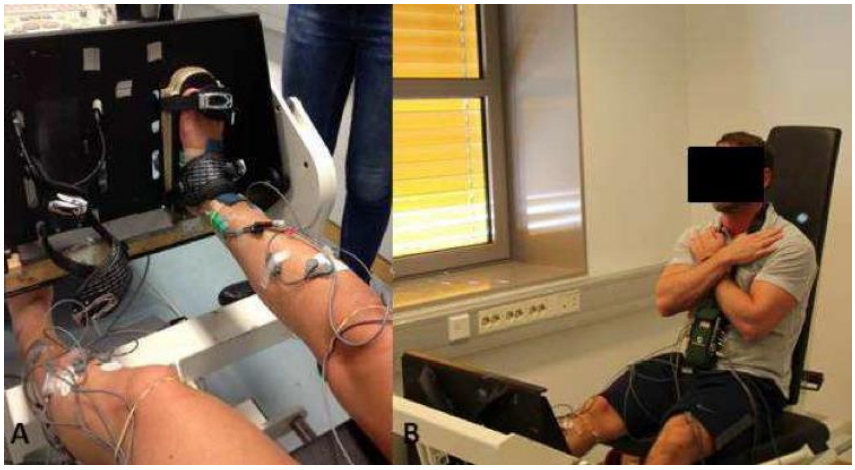


Figure 9: Positioning in the isometric ergometer. A.) Securing of the test foot and positioning of the relaxed foot. B.) General positioning (right) with straight knee, upright back and arms crossed over the chest.

The subjects were provided with both visual and oral feedback on their performance. They were instructed to follow a loading curve displayed on a computer screen to ensure constant increase in muscle force. The subjects performed three trials on each scanning position. A trial was approved when maximal force was reached, US scanning was satisfactory and when the force increased near-linearly (from visual inspection). There was 2 minutes break between each trial.

4.4.6 ROM (ROM)

Maximum dorsiflexion ROM was measured in the isokinetic dynamometer. The subjects were secured to the chair with 65˚ hip angle (0˚ corresponding to anatomical position). Seat height and back angle was standardized, but individual adjustments noted on the accommodation testing were set up to match the mediolateral axis of the ankle joint with the rotational axis of the dynamometer. Also dynamometer height was

adjusted to ensure an extended knee position. With this set-up the subjects felt no sense of stretching in the calf or hamstrings whilst sitting in the initial position.
The foot, strapped on the footplate, was manually and slowly moved from a 25˚ ankle angle in plantarflexion to maximal dorsiflexion. The same experimenter for all subjects performed this procedure. The subjects were instructed to signal the experimenter to stop the movement once they had reached their maximum limit of stretch tolerance, and the corresponding joint angle was admitted as maximum ROM and inserted into the dynamometer computer software. The subjects were asked to state their level of discomfort or pain caused by the stretch on a visual analogue scale(VAS). Maximum ankle dorsiflexion angle was recorded and used in other tests when required. The maximal ROM was determined as the difference in degrees between the absolute zero and the maximum tolerated in dorsiflexion (Figure: 10).

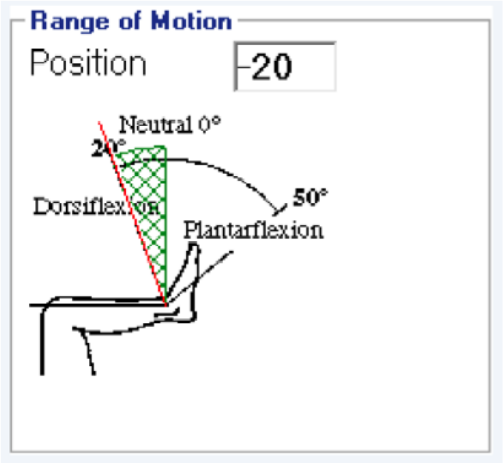


Figure 10: Defining ROM. ROM was tested with at straight knee. (Print screen Humac Norm Software, Human Asessment Computer, HUMAC 2009, Version 10.000.0026, Computer Sport Medicine, Stoughton, MA, USA)

4.4.7 Passive movement

After the IRC the subjects were given a 3-minute break to ensure that muscular or cognitive fatigue would not interfere with the test results. Then they were seated in the isokinetic dynamometer for recording of passive torque.
The following passive motions were executed in the following order (positive angles indicating plantarflexion):

- 4 short passive motions from -5° to 10°
- 6 passive motions from 0° to maximum dorsiflexion

From the short passive motions passive torque and ultrasound video of calcaneus rotation and soleus MTJ excursion was obtained, and would later be used for corrections for ankle rotation. Passive torque and ultrasound videos were recorded for 6 repetitions of continuous passive motions from 25 degrees plantarflexion to maximal dorsiflexion.
For the torque at max ROM trials 2 ultrasound videos were taken of Gastrocnemius Medialis MTJ, Soleus MTJ and Gastrocnemius Medialis muscle architecture. Data from which will not be reported in this particular thesis.

4.5 Anthropometric Measurements

Height was measured with a height scale (Seca Gmbh, Model 217, CITY, Germany) and weight was measured with a digital weight scale (Seca Gmbh, Model 877, CITY, Germany).
All lower limb measurements were conducted whilst the subject was standing, using a measuring tape. Lower limb length was measured from trochanter major to the floor, and leg length was measured from the lateral epicondyle of the knee to the calcaneus. Identification of anatomical landmarks was made by palpation.

4.6 Mechanical properties

For assessment of tendon mechanical properties, elongation of the free AT was measured with ultrasound during isometric ramped contractions of the plantarflexor muscles. Reeves et al. (2003) have shown this method to be reliable and this has since been utilized by a number of research groups (Ito et al., 1998; Fukunaga, Ito, Ichinose, Kuno, Kawakami, & Fukashiro, 1996; Maganaris, 2002; Maganaris & Paul, 2002; Bojsen-Møller et al, 2003).

4.7 Electromyography (EMG)

EMG data were recorded from Soleus GM and GL during passive stretching to ensure that these muscles remain silent. EMG was also recorded from the tibialis anterior to estimate antagonist co-activation (see details below). In preparation of skin for attachments of EMG surface electrodes (Ambu, Blue Sensor N, Ballerup, Denmark) the skin was cleaned with isopropanol and gently rubbed with a very fine sanding paper in order to ensure optimal skin impedance for recording of EMG signals. EMG electrodes were placed on GM, GL, SOL, and TIB with a reference electrode on the lateral part of the tibial tuberosity.

4.8 Ergometers

For both active and passive tests an isokinetic dynamometer was used (HUMAC ®/NORM ™ Model 770, Computer Sports Medicine, Inc. CSMI, Stoughton, MA, USA). For passive tests and isometric contractions a machine built especially for these purposes was used, and will hereafter be referred to as the isometric ergometer GYM2000, Norwegian School of Sports Sciences, Oslo, Norway).

4.9 Ultrasound (US)

To collect videos and still images of the muscle-tendon system of the triceps surea a B-mod ultrasound system (HD11XE, Phillips, Bothell, WA, USA) with a 50-mm wide linear transducer (L12-5) was used. Video was taken with a depth of 3 - 8 centimeter (cm). Length of the free Achilles tendon (AT_f), as well as the tendon cross sectional area (CSA) of the proximal, distal and mid portion of the AT_f, was determined by ultrasonography.

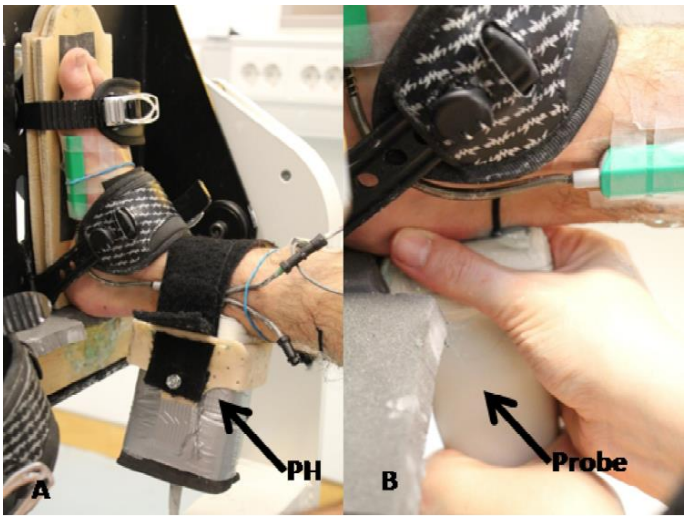


Figure 11: The US probe while scanning in the sagittal plane. A.) The probe holder (PH) fastened to the lower leg for scanning of Soleus myotendinous junction. B.) Probe in place for scans of the Achilles tendon calcaneus insertion. The PH could not be fastened, thus this had to be done manually.

The probe was fastened to the limb using a custom-made probe holder during muscle contractions and passive motion. Echo absorbing tape (Figure 12) served as a reference marker during ultrasonography in active and resting tasks and was placed distal to the Gastrocnemius Medialis myotendinous junction (GM) or Soleus myotendinous junction (SOL MTJ) during isometric contractions, and proximal to the MTJ of the respective muscles during continuous passive movements.

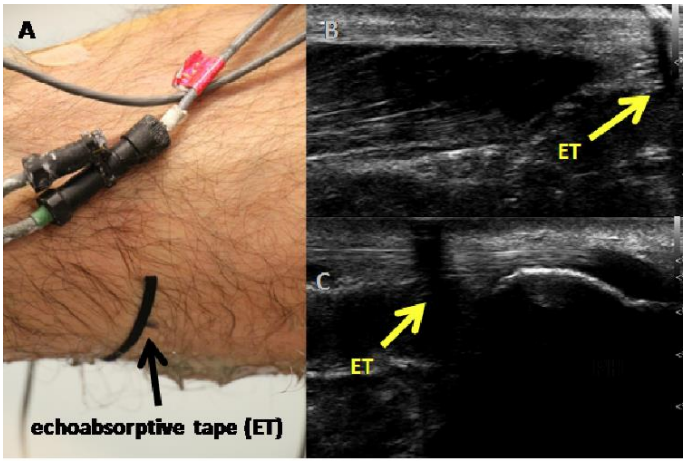


Figure 12: A.) Echo absorbing tape placed on the skin surface. B. / C. Echo absorbing tape appears clearly in the ultrasound videos.

To correct for the degree of ankle rotation during the testing, an electrical goniometer (Noraxon DTS Telemyo System, Inc., Scottsdale, AZ, USA) was fastened securely on the medial side of the foot and ankle (Figure: 13).

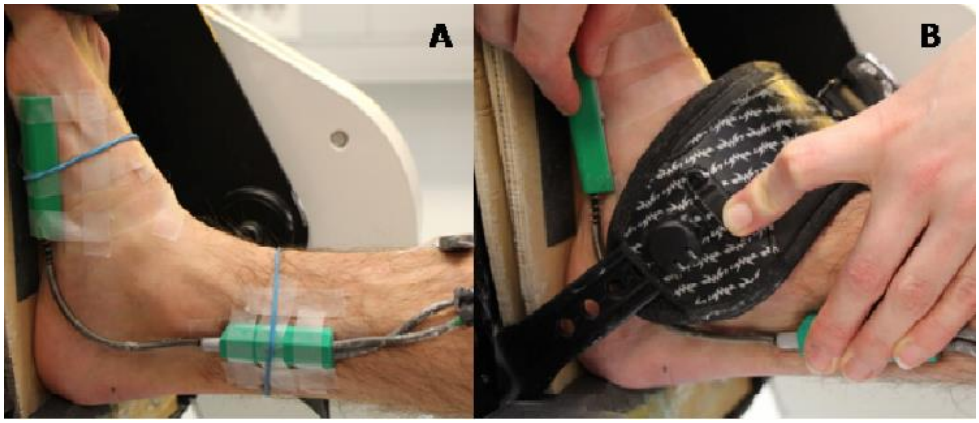


Figure 13: Positioning of the electrical goniometer. A: The goniometer was attached medially on the foot and ankle with strong surgical tape. B: Foot strapping did not interfere with the sensor axis of the goniometer.

4.10 Synchronization of data

Force data, EMG data and ultrasound videos were then synchronized by the aid of a wireless receiver (Mini-receiver for TeleMyo G2, Noraxon, Inc., Scottsdale, AZ, USA). For data synchronization all data was transmitted and stored with a personal computer interface (MyoResearch XP Master Edition 1.08.17, Noraxon Inc., Scottsdale, AZ, USA). Signals for synchronization of all recording units and US videos were produced by a generator (function generator, GwinStec, GFG-8215A, Good Will Instrument Co., Ltd, Tucheng City, Taiwan). EMG activity and signals from the goniometer were simultaneously transmitted through a wireless telemetric transmitter (16channel TeleMyo 2400 G2 Telemetry System, Noraxon, Inc., Scottsdale, AZ, USA) to a wireless receiver (Mini-receiver for TeleMyo G2, Noraxon, Inc., Scottsdale, AZ, USA). Force data, EMG data and ultrasound videos were then synchronized by the aid of the wireless receiver and from there to the PC interface (Figure 1). Data from the Isometric ergometer and the dynamometer were transmitted by aid of an analogue input board (TeleMyo 2400 GT receiver, Noraxon Inc., Scottsdale, AZ, USA). Ultrasound videos were transmitted to the PC interface by a DV converter (ADVC-55, EFC-152265, Canopus Co., Ltd, Kobe City, Hyogo, Japan) and they were also stored on the US machine. EMG, force data and ultrasound videos from the ramped contractions, passive

motions, isometric- and isokinetic contractions were stored with Noraxon software (MyoResearch XP Master Edition Version 1.08.17, Noraxon Inc., Scottsdale, AZ, USA) on a computer. Ultrasound material was stored on the ultrasound unit.

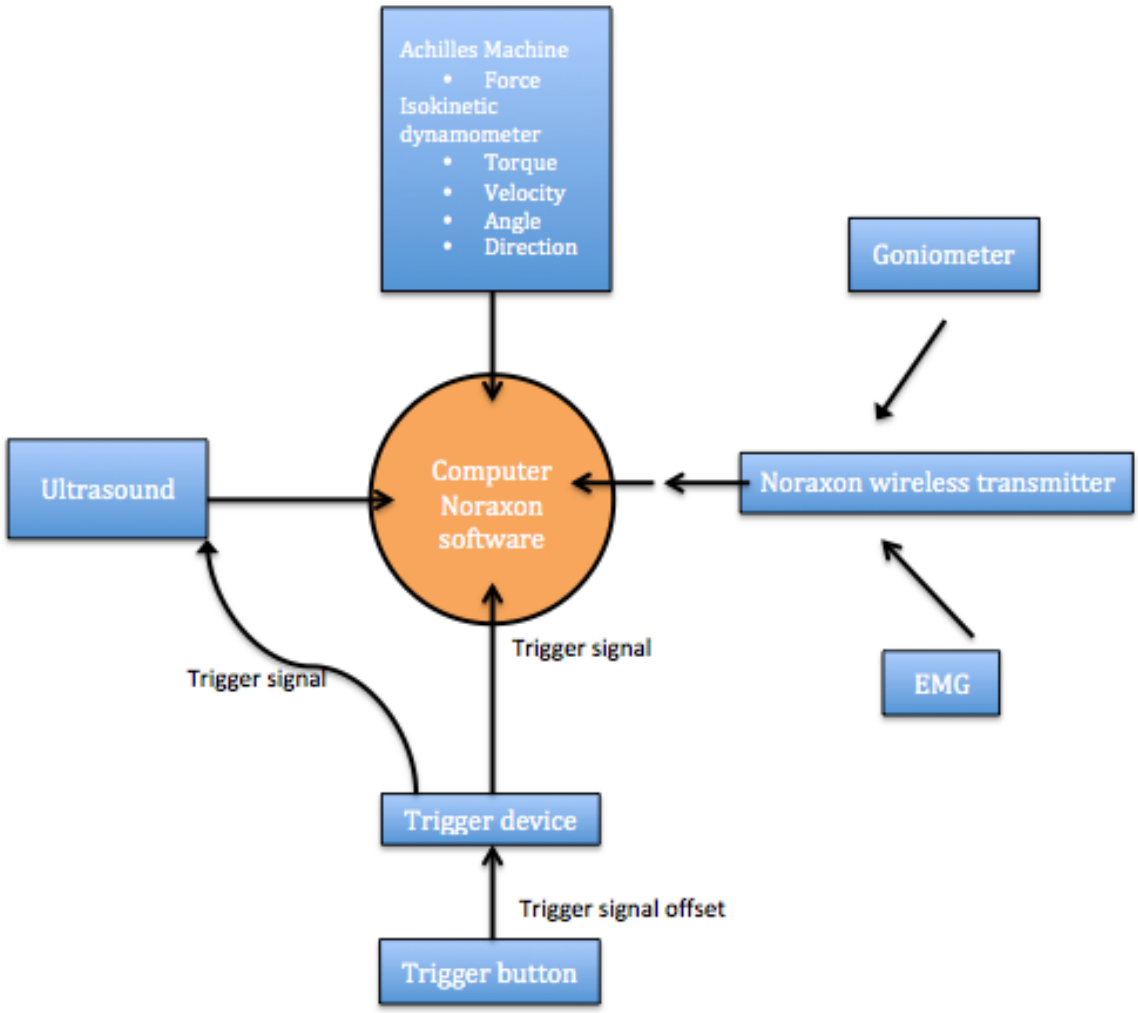


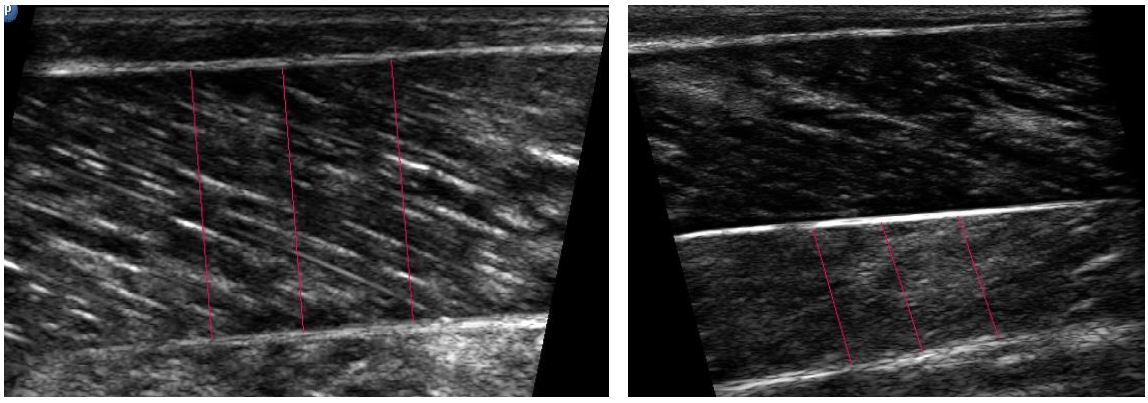
Figure 14: Overview of data synchronization

5.0 Data analyses

5.1 Muscle architecture

All measurements of muscle and tendon morphological properties were analyzed and measured with Fiji ImageJ (1.0) (Tendon length) and ImageJ (1.46r, National Institutes of Health, Austin, TE, USA) (muscle thickness, fascicle length, pennation angle and tendon CSA).

Muscle thickness was measured on both GM and SOL ultrasound images. 3 measurements were made: one on the center of the image and two on either side approximately 1 cm apart form the mid measurement. Measurements were from the upper to the lower aponeurosis, and perpendicular to both aponeuroses.



A B

Figure 15: Muscle thickness analyses. A.) Analysis of Gastrocnemius Medialis thickness. B.) Analysis of Soleus thickness. The 3 measurements for each muscle belly were averaged.

5.2 Tendon length

The collected US images were combined and tendon length was analyzed and measured with Fiji ImageJ 1.0. A selection tool was used to manually trace the lower edge of the Achilles tendon. Length measurements were made from the GM MTJ and from SOL MTJ, to the distal insertion of the Achilles tendon on the calcaneus. 3 measurements were taken from GM and SOL MTJ and the averaged.

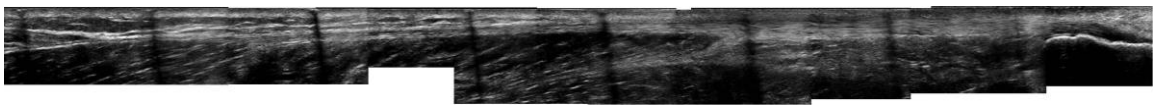


Figure 16: Ultrasound images combined in the analysis software for measurement of Free Achilles tendon length and Gastrocnemius tendon length.

5.3 Fascicle length

A fascicle visible through the whole muscle belly was chosen for fascicle length analysis. A selection tool was used to manually trace the fascicle and 3 measurements were made and averaged.

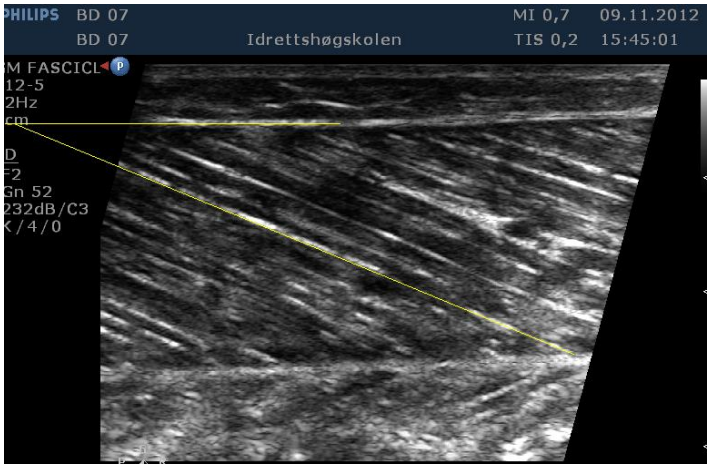
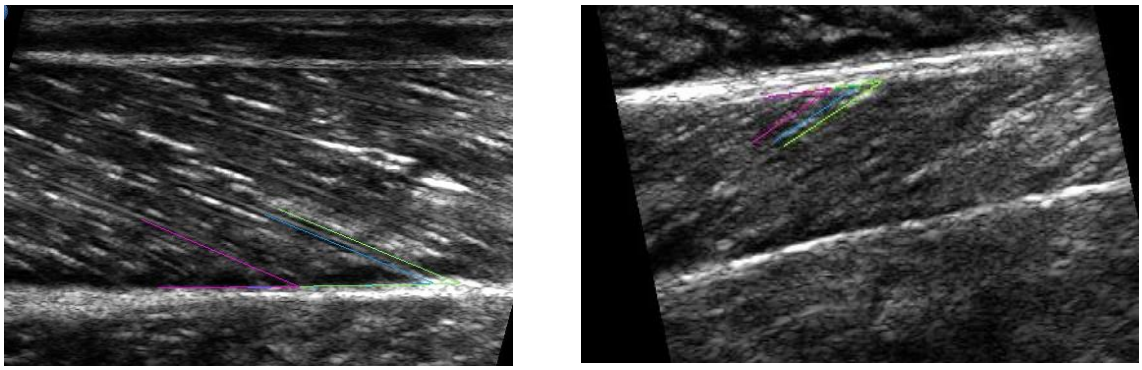


Figure 17:Measurement of muscle fascicle length. The aponeuroses of the muscle belly were extrapolated in the cases where the fascicle was not visible throughout the entire muscle belly.

5.4 Pennation angle

For GM pennation angle measurements were made on the lower aponeurosis and for the SOL pennation angle measurements were made at the upper aponeurosis. A fascicle with clear visibility at least for the length of 1/3 of the muscle thickness and inserted on the respective aponeuroses was chosen. An angle selection tool was used to draw on top of the fascicle and on the aponeurosis edge, before 3 measurements were made and averaged.



A **B**

Figure 18: Analysis of muscle architecture on US images. A.) Gastrocnemius fascicles were measured on the edge of the deeper aponeurosis. B.) Soleus fascicles were measured on the upper aponeurosis.3 clearly visible fascicles were analyzed and averaged.

5.5 Tendon CSA

Tendon CSA was measured with a video analysis program (see..above). An image mid-, proximal and distal CSA with the clearest outline was used for analysis. Further, 3 measurements were made on each image and averaged.

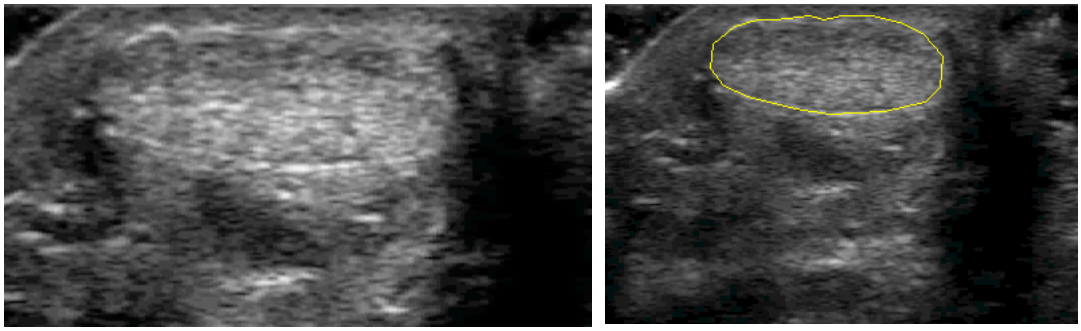


Figure 19: Left: Zoomed view of the Achilles tendon, scanned with the probe in the transverse plane. Right: Manual outline of the area for interest.

5.6 Tendon elongation

Tendon displacement during resting and active motion trials was analyzed from ultrasound videos stored in the Phillips/Noraxon software as AVI files. The displacement of proximal (SOL MTJ and GM MTJ) end and insertion (calcaneus) was measured on ultrasound videos utilizing a video tracking software (Tracker Video Analysis and Modelling Tool 4.62, Open Source Physics, Douglas Brown, 2012). The coordinates from the external marker and respectively GM MTJ, SOL MTJ or AT calcaneus insertion were automatically tracked throughout the whole isometric ramp contraction. The tendon displacements were corrected for possible probe movement. For this purpose the external marker was tracked (Maganaris & Narici, 2005; Arampatzis et al., 2007) the displacement of the marker was subtracted from the total tendon displacement. Displacement at the distal end was also corrected for ankle rotation by synchronizing data from passive motion trials with ankle joint rotation during isometric ramp contractions recorded by the goniometer.

5.7 Tendon force

All data processing was done in MATLAB (R2013a, The Math Works Inc.). Tendon force was calculated from the torque measurements obtained from the isometric ergometer force cell, by the use of the internal moment arm of the Achilles tendon. In

line with previous literature, 43 mm was used for the moment arm (Kongsgaard et al., 2011). Antagonist co-contraction was corrected for presuming a linear relationship between force and EMG amplitude (Magnaris et al., 1998; Bojsen-Møller et al., 2004). Force was calculated with the following formula:

$F_t = ((T_{erg} + T_{tib}) / M_i$

F_t = *Tendon Force*
T_{erg} = *Torque recorded form the isometric ergometer*
$Torque_{tib}$ = *Tibialis anterior Co-activition*
M_i = *Internal moment arm*

EMG signals were smoothed with a Butterworth band-pass filter and a root-mean square equation (100 ms). A linear relationship between force and EMG amplitude was assumed in order to correct for tibialis anterior co-activation. Further, we calculated how many present of MVC the corresponding EMG signal in the ramp contraction was in Newton (N). Max torque and corresponding EMG was highest mean over 500 ms. The force was then added to the isometric ramp contraction force-curve. The force was then normalized to calf length.

5.8 Mechanical and material properties

After calculation of tendon force and tendon deformation, a force-elongation relationship was made and fitted to a 2nd order polynomial equation. Tendon stiffness was calculated at in 80-100% of the force-deformation curve.
Relative tendon stiffness was calculated a maximal common force level of the weakest subject which in this study corresponded to 2300 N and absolute stiffness at individual maximal force was calculated. Young's modulus was calculated for both absolute and relative stiffness, by multiplying tendon stiffness values with the ratio between the start length of the tendon and mean CSA.

5.9 Statistics

Differences between the elite professional dancers and the control group were analyzed with a two-tailed unpaired t-test, where the alpha-level of significance was set to 0.01 or 0.05. Results are presented as mean ± standard deviations (SD). All statistical analyses were executed in Excel (Microsoft Excel 2011 Inc., Microsoft) or Graph Pad Prism (Graph Pad Software, San Diego, CA, USA).

6.0 Results

6.1 Anthropometric measurements

There were no significant differences between the dancers and the control group (p-value 0.05).

Table 2: Anthropometric measurements and subject characteristics represented as means ± standard deviation.

	Dancers	Controls
Age (years)	26.3 ± 4	23.4 ± 1
Weight (kg)	60.6 ± 7	60.4 ± 7
Height (cm)	168 ± 6	168 ± 7

Leg Length (cm)	**Trochanter major to floor**	**Lateral epicondyle of the knee to posterior calcaneus**
Ballet Dancers	85.25 ± 5.5	44.1 ± 2.18
Controls	85.15 ± 5.54	44.3 ± 2.22

6.2 Range of Motion

The figure shows that dancers display significantly larger maximal ROM (40.1 ± 6.7) compared to controls (17.4 ± 9.17) ($P < 0.01$).

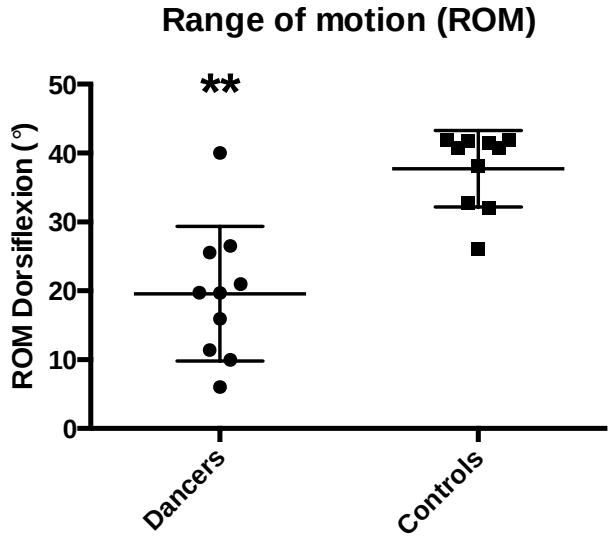


*Figure 20: Comparison of ROM between dancers and controls **Significant difference with P<0.01*

6.3 Muscle Architecture

In the present thesis muscle architecture is represented by pennation angle, GM thickness, SOL thickness and relative fascicle length. Fascicle length was normalized to leg length (mm from lateral epicondyle of the knee to posterior calcaneus).

Pennation Angle (°)

There were no significant differences found in Soleus or Gastrocnemius Medialis pennation angle in Dancers (GM: 23.7± 1.6 / SOL: 22.1 ± 4.6) compared to Controls (GM: 25±2.4 / SOL: 24.5 ± 5.2).

Muscle thickness (mm)

GM muscle thickness was significantly greater in dancers (21.7 ± 2) compared to controls s (17.7 ± 2) ($P < 0.01$). No significant difference was found in SOL thickness when dancer (17.6 ± 2) were compared to controls (17 ± 4).

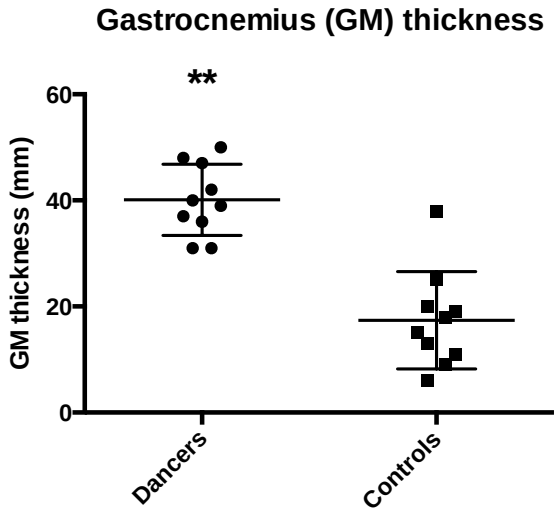


Figure 21: Comparison of GM muscle thickness in dancers and controls
***Significant difference with P<0.01*

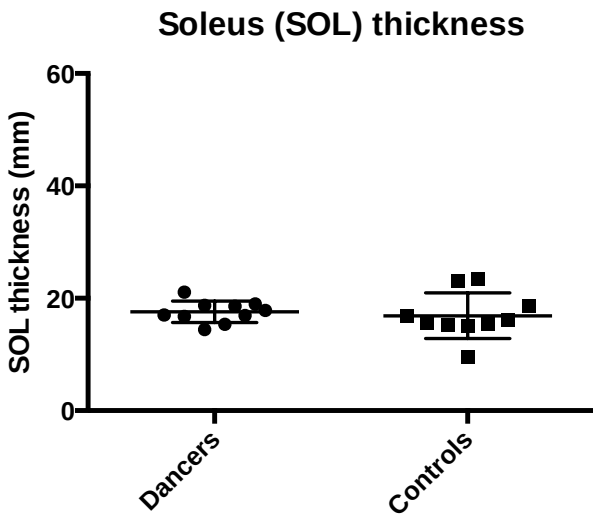


Figure 22: Comparison of SOL muscle thickness in dancers and controls.

Fascicle length (mm)

Fascicle length normalized to leg length was significantly ($P < 0.01$) longer in dancers (12.8±06) compared to controls (9.5 ± 0.3). There was a small difference ($P < 0.05$) in the opposite direction with longer relative SOL fascicle length in controls (9.4 ± 1) compared to dancers (8.4 ± 0.8).

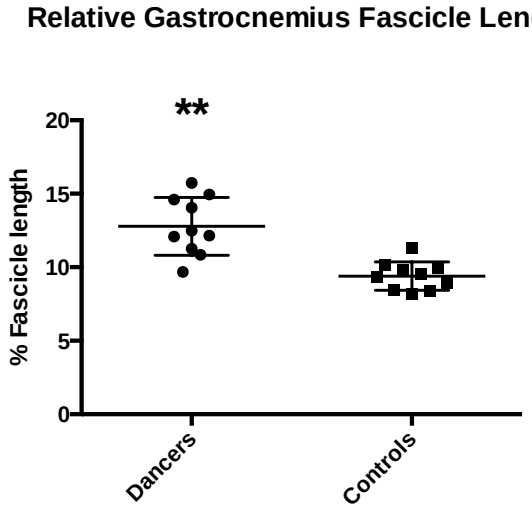


Figure 23: Comparison of relative GM fascicle length in dancers and controls
***Significant difference with P<0.01*

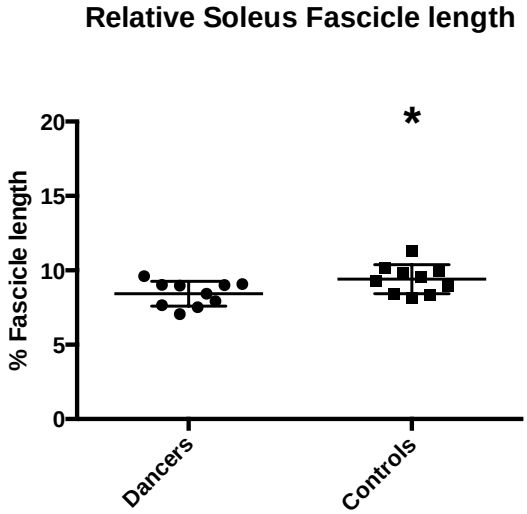


Figure 24: Comparison of relative SOL fascicle length in dancers and controls
**Significant difference with P<0.05*

6.4 Tendon Morphological Properties

In the unloaded position dancers demonstrate significantly longer GM tendons compared to controls (dancers: 207 ± 33 mm, controls1: 66.8 ± 10 mm) ($P < 0.01$). No differences between dancers and controls were found in the unloaded free Achilles tendon. In the 0°- position dancers had significantly longer GM tendons (220 ± 32 mm) compared to controls (175 ± 19 mm), further free Achilles tendon in 0°-position were also longer in dancers (81.7 ± 33.7 mm) in comparison to controls (55 ± 25.2 mm).

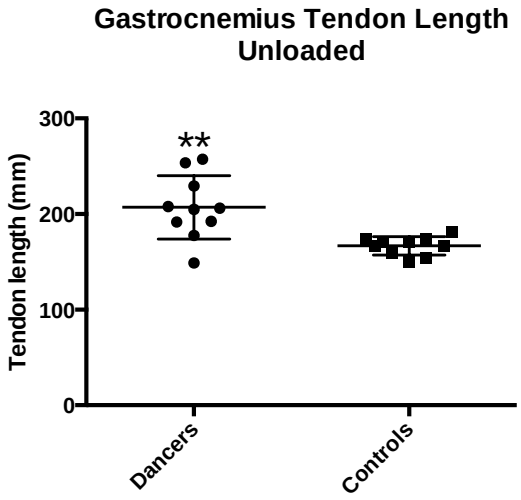


*Figure 25: Comparison of GM tendon length between dancers and controls, measured with feet resting freely out side a bench and subject in unloaded position.**Significant difference with P<0.01*

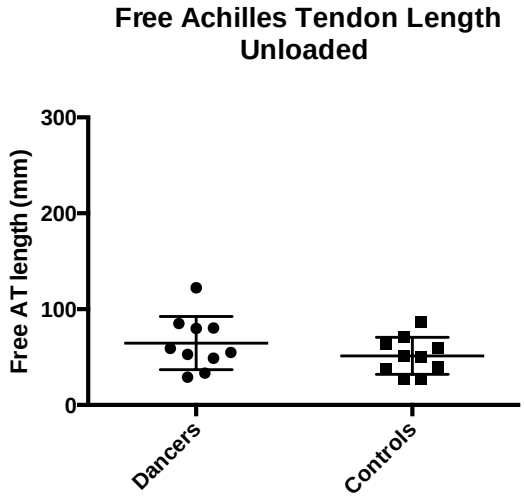


Figure 26: Comparison of free AT length between dancers and controls measured with feet resting freely out side a bench and subject in unloaded position.

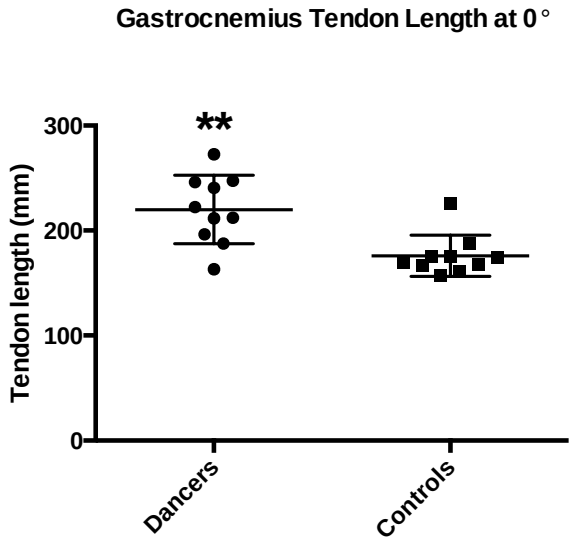


*Figure 27: Comparison of GM tendon length between dancers and controls. **Significant difference with P <0.01*

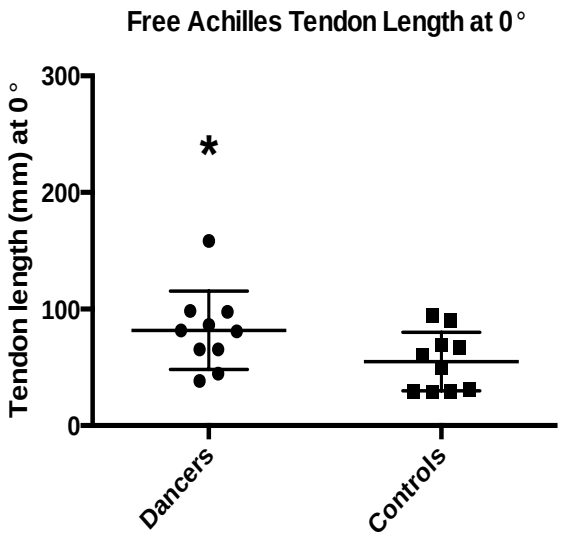


*Figure 28: Comparison of Free AT length between Dancers and Controls. *Significant difference with P <0.05*

6.4.1 Tendon Cross-Sectional Area (CSA mm^2)

No significant differences were found in tendon CSA at either the proximal (dancers: 62.2 ± 14, controls: 62.10 ± 25), distal (dancers: 74.8 ± 15.3, controls: 73.2 ± 19.7) or mid (dancers: 59.4 ± 11.5, controls: 52.96 ± 7.8) portion of the free AT.

6.5 Mechanical Properties

Significant differences at -10° were found between dancers (15.4 ± 6.4, n = 10) and controls (24.5±9.2, n = 9) ($P < 0.01$). -10° was the highest common dorsiflexion angle among dancers and controls taken together.

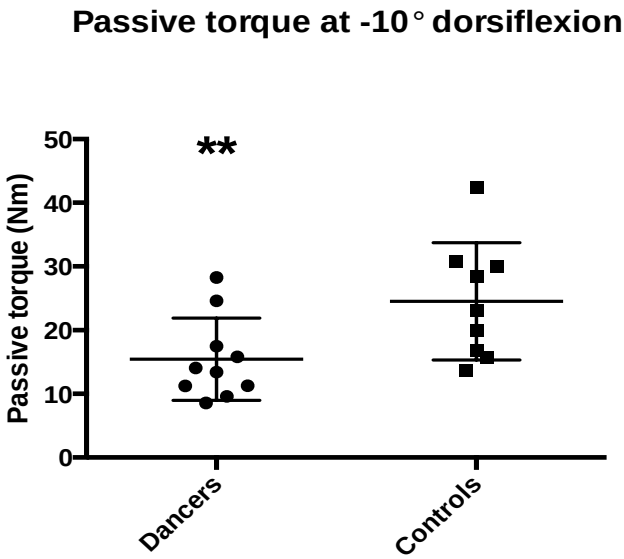


*Figure 29: Comparison of passive torque at -10° dorsiflexion between dancers and controls **Significant difference with P<0.01*

Force - elongation relationship

Figure 30 and 31 represents the force-elongation relationships from isometric ramped contractions. There were no significant differences from force level 0-2500N. In the force-elongation relationship of the free AT in dancers and controls. However, elongation in dancers and controls was significantly different from 100N-2500N with $P<0.05$ and from 600N-2500N with $P<0.01$. Further, no significant differences between dancers or controls were found from force level 0-2500N. However, elongation was significantly different from 200N-2500N with $P<0.05$ and from 500N-2500N with $P<0.01$

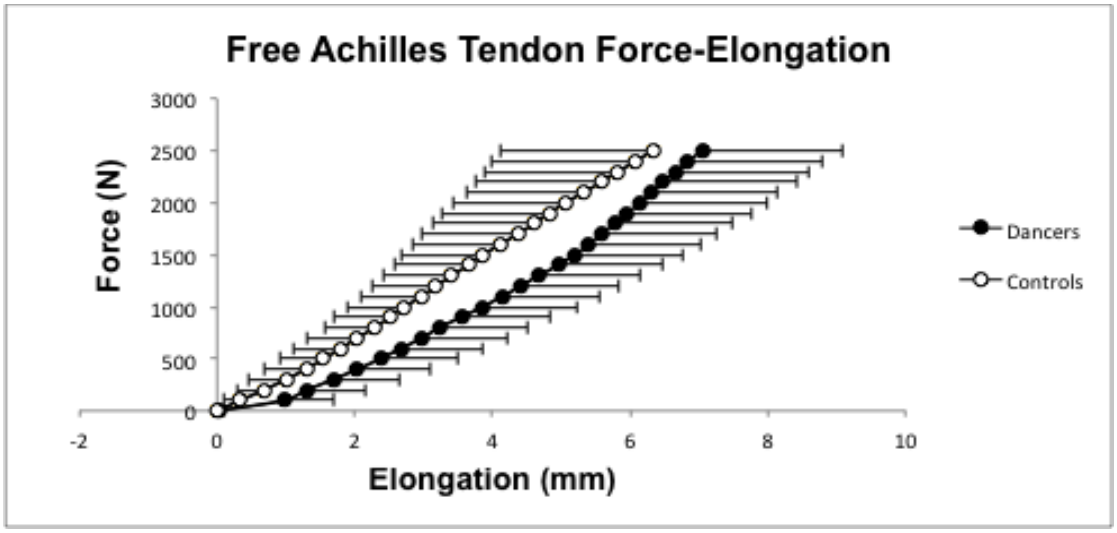


Figure 30: Relationship between force (N) and Free Achilles tendon elongation (mm) in dancers (n=10) and controls (n=9). Values are means for 3 trials for each subject.

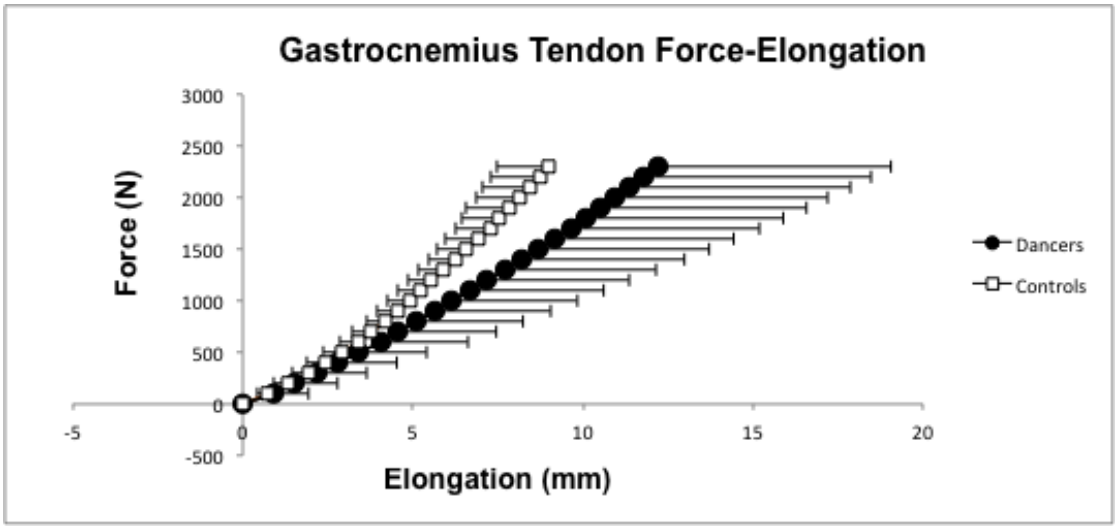


Figure 31: Relationship between force (N) and Gastrocnemius tendon length (mm) in dancers (n=10) and controls (n=9). Values are means for 3 trials for each subject.

Table 4: Mechanical and material properties for the Dancers and the Controls. Stiffness absolute and Young's modulus absolute are absolute values calculated from peak force from ramped isometric contractions in plantar flexion. Stiffness relative and Young's modulus relative are values calculated from the greatest common force level at 2300 N (n=9 per group). *Significant difference with P<0.05.

	Dancers (n=10)	**Controls (n=9)**	**P-value**
GM tendon Stiffness absolute ($N \cdot mm^{-1}$)	324 ± 198	425 ± 167	0.262
GM tendon Stiffness relative ($N \cdot mm^{-1}$)	297 ± 168	382 ± 133	0.245
Free AT Stiffness absolute ($N \cdot mm^{-1}$)	484 ± 195	610 ± 360	0.371
Free AT Stiffness relative ($N \cdot mm^{-1}$)	439 ±146	570 ± 317	0.279
GM tendon Elongation (mm)	12 ± 7	9 ± 2	0.208
Free AT Elongation (mm)	8 ± 1	6 ± 2	*0.038
GM tendon Strain (%)	6 ± 4	5 ± 1	0.63
Free AT Strain (%)	11.3 ± 4.6	12 ± 5.2	0.779
GM tendon Young's modulus absolute (Gpa)	0.89 ± 0.43	0.85 ± 0.26	0.85
GM tendon Young's modulus relative (Gpa)	0.81 ± 0.38	0.74 ± 0.19	0.625
Free AT Young's modulus absolute (Gpa)	0.41 ± 0.15	0.37 ± 0.16	0.63
Free AT Young's modulus relative (Gpa)	0.37 ± 0.15	0.34 ± 0.13	0.742

7.0 Discussion

In the present thesis a group of elite professional dancers (24 ± 5 years of classical ballet training) was compared to a matched control group, investigating architectural, morphological, material and mechanical properties of the triceps surae MTU *in vivo* and noninvasively with ultrasound.

In agreement with the Hypothesis 1, dancers displayed larger ROM and lower passive torques at the highest common dorsiflexion angle compared to controls. Further, dancers showed longer GM muscle fascicles and greater GM muscle thickness compared to active controls, which confirms Hypothesis 2 and contradicts other findings from human stretching studies. Furthermore, the results demonstrate that the dancers had longer free AT and longer GM tendons in both the 0°- position and in the unloaded conditions compared to controls. In the free AT and GM tendon there was no difference found in strain between dancers and to controls, and the free AT displayed larger deformation during ramped isometric contractions compared to controls (Table 4 and Figure 30). The force-elongation relationship from isometric ramp contractions illustrates the difference in the entire triceps surea MTU's ability to deform, where the controls display a display a steeper slope than dancers. This was expected, due to significantly longer free AT in dancers compared to controls.

7.1 Differences in ROM

Enhanced ROM following stretching exercise has been shown with various long-term durations of stretching exercise interventions (Kubo et al., 2002; Wessling et al., 1987, Nakamura et al., 2012; Magnusson & Weppler, 2012; E Lima et al., 2014; Magnusson et al., 1996b; Ben & Harvey, 2010). Amongst all the factors influencing ROM, neural factors might be related to the daily emotional status of the subjects. If neural mechanisms are affecting ROM, the outcomes may be divergent. In studies like the present thesis it is impossible to blind the subjects from knowing that they are participating in a stretching study and testing

Studies have shown that the influence of cortical activity, in this context emotional state, modulates human pain reactivity, and that the anxiety of pain may amplify the

sensation of induced pain (Dougher et al., 2000; Rhudy & Meagher, 2000), which way well affect the maximal ROM results in these subjects. Nonetheless, there is a robust difference ($P<0.01$) in measured maximal ROM between dancers and controls. With the high stretching exercise volume, frequency and intensity practiced by dancers from a very young age (7 ± 4 years) it there are clear indices that long-term stretching exercise increases ROM. It may be that the increased tolerance to stretch could be due to lowered Hoffmann reflex activity (Nielsen et al., 1993) and reciprocal inhibition, autogen inhibition (myotatic reflex).When considering ROM alone it remains open to question which parameters did in fact change to allow for ROM to increase. Significant increases in ROM in itself do not provide information regarding possible adaptations in properties of the MTU system. The sensory theory states that maximal ROM is increased solely because of increased tolerance to stretch (Weppler & Magnusson, 2012; Alter, 2004). When contemplating differences in loading on the MTU long-term, one would expect living biological tissue to adapt to the stimuli and if increased tolerance to stretching alone was the only mechanism for increased ROM, one could possibly expect to see an increase in ROM also in the control-leg in a stretching exercise intervention, due to the fact that neural adaptations can be transferred to the contralateral side (Nelson, 2012).

7.2 Tendon morphological properties

The main findings regarding tendon morphology (Figure 25-28) show 20% longer GM tendons and 32.5% longer free AT in dancers in the 0º- position compared to controls (GM tendon $P < 0.01$. Free AT < 0.05). Also in the unloaded position the GM tendon was longer with 19.5% in Dancers ($P <0.01$) compared to controls, but no length difference was found in the Free AT. Results from dancers and controls taken together shows that the individual variation in tendon lengths at 0º-position (GM tendon 156 – 273 mm. Free AT 29-158 mm) with relatively large standard deviations in both groups. This is in line with anatomical studies of the Free AT, where tendon lengths for 3 to 11 cm have been measured (Pierre-Jerome, Moncayo, & Terk, 2010; Nickisch, 2009). For the GM tendon the results are also coherent with the literature where GM tendon length of 225 ± 20 mm have been reported (Magnaris & Paul, 2002). The enormous length differences between subjects may be explained by genetic determinations (Alter, 2004).

The robust differences in tendon lengths between dancers and controls found in this study are interesting, however, human studies have yet to show that static stretching

induce length changes in either the Free AT or the GM tendon. Adaptations to long-term stretching have been explained by the aforementioned increased tolerance to stretch or by adaptations in the muscle. It has been argued that stretching does not increase the elasticity, that is the series elastic components in the tendon, but it does affect the connective tissue in parallel with the muscle, the parallel elastic components (Kubo et al., 2002). Because of the cross-sectional design in this study we cannot argue that long-term stretching is the cause of the longer Free AT and GM tendons seen in dancers compared to controls. Nonetheless, studies showing loading induced differences in tendon CSA (e.g. Couppé et al., 2008) support the concept of morphological plasticity of this tissue. Couppé at al. (2008) found robust side-to-side differences in patellar tendon CSA of elite fencers and badminton players, who habitually load their legs differently due to sport-specific demands. Also stretching intervention studies of animal models showed morphological changes in tendon length. Warren et al. (1976) showed increased and sustained rat-tail tendon length after stretching exercise. The rats stretched either stretched low-load, long duration (50 min) or vigorous, short duration (5 min). How long a static stretch is held and the magnitude of the load applied to the tendon seems to have had effects on tendon length change in the rat-tails. While we cannot be certain of the load magnitude or the exact duration the Dancers apply to their daily stretching exercise, the authors field experience (>25 years) is that the duration of a static stretch can vary between 1 to 10 minutes, with 1 to 4 repetitions. In the study of Warren et al. the 5 minute- stretch is called *short duration*, however compared to stretching durations (4s-60s) in human studies 5 min is rather long duration, and it seems dancers in general stretch more like animal models than human research subjects. Therefore, previous long-term stretching exercise studies may have been insufficient in durations, load magnitude, or both to produce a detectable tendon length response to stretching exercise. In addition to their daily stretching exercise, dancers also perform movements involving joint angles that are within the limits of their maximal ROM.

Studies have shown the tendon length has implications for the MTU function due to the stretch-shortening cycle capacity, where the tendon stores and releases energy in movement tasks like running and hopping (Lichtwark & Wilson, 2006a; Biewener et al., 1998). A recent study has also reported differences in GM tendon length between one sport-specific group and a matched, active control group. Sano et al. (2012) used ultrasound to investigate GM tendon length in 10 male, Kenyan elite middle- and long

distance runners, and compared them to 10 Caucasian controls. Further, they used EMG and kinematics to investigate muscle –tendon interaction during maximal hopping, assessing stretch-shortening cycle and active fascicle length changes. Longer GM tendons were found in the Kenyan runners (264.2 ± 24.5 mm) compared to the control group (196.6 ± 12.8 mm), and with the larger shortening to stretch ratio of the GM tendinous tissues in the Kenyan runners this could imply that the GM MTU is optimized for efficient energy storage and release of elastic energy (Sano et al., 2012). Longer GM tendons and better hysteresis compared to controls may well be one of the multiple factors that have made East Africans dominate in international track events. If these results are transferable to Kenyan and/or Caucasian women is not known. Nonetheless, the difference in GM tendon length found by Sano et al. is similar to the present results. While the dancers are a sample of different nationalities (American, Norwegian, Swedish, White Russian, French and English), it may be possible that genetic endowment in the form of a long tendon, thus natural selection has brought the subjects to pursue dance exercise early in life. With the amount and physical demand of running-like, hopping and bounding movements in the ballet syllabus it would most definitely be advantageous to with long GM- and Free Achilles tendons.

There was no difference in tendon CSA at either the proximal, - mid- or distal region of the Free Achilles tendon. This is not surprising. Even though the dancers load their plantarflexors habitually with both strength- and stretching exercise, they were after all compared to active controls with a training history and training status that involve strength training or other types of activity placing a substantial load on the MTU. Strength training studies have previously shown that tendon CSA is associated with muscle hypertrophy (Reeves et al. 2003; Kongsgaard et al. 2007). Therefore, tendon CSA was not expected to be different between the groups.

7.3 Differences in muscle architecture

Some of the main findings in this thesis are the differences in normalized muscle fascicle length, partly confirming Hypothesis 2. The largest difference ($P<0.01$) was found in GM, where dancers displayed 26% longer GM muscle fascicles compared to controls. Considering the present results, and what we have learned from animal stretching studies (Warren et al., 1976; Tabary et al., 1972; Stolov et al., 1970), the

present results might fit with the theory regarding the addition of sarcomeres in series. The dancers are stretching their MTU systems to the maximal ROM that they can tolerate, with a high frequency and long duration (1-10 min), and this is has been their conduct for years. Therefore, one cannot ignore that adding of sarcomeres in series to adapt the muscle to a functional ROM might be the case for such a population. Even if the magnitude of loading when the Dancers stretches daily is not known, the durations of which they stretch are similar to those of the study by Warren et al. (1976). Thus, in the long-term perspective the stimuli form both the static stretching practiced specifically to preserve or increase ROM together with the dynamic stretching in the dance training and stage work might be stimuli of sufficient size for sarcomere adding to commence.

On the other hand the larger muscle thickness seen in the Dancers' GM, might result from hypertrophy due to the strength training. If there is no difference in muscle thickness between Dancers and Controls this advocates for adding of sarcomeres in series in response to stretching training, as seen in the animal studies. When strength training is mentioned in relation to the dancers, it is not meant in the conventional way. E.g. still to date very few female dancers lift weights, and the strength training they are exposed to is from transporting their own limbs and their bodies from one position to the next at different speeds (lifting their legs slowly, kicking and jumping) and usually with many repetitions (>10). In other words female dancers practice strength training in low velocities with low to moderate intensity, and power training close to fatigue. Cross-sectional studies reported longer muscle fascicles in wrestlers and sprinters compared to controls. In such sports traditional heavy weight strength training is practiced to a large extent (Raastad et al., 2010). Differences in muscle fascicle length could be an adaptation to the habitual loading of the muscles. Dancers muscles have possibly adapted to an optimum size in order to serve the demands to force-velocity and angle-velocity relationship that is adequate for their type of physical performances.

Muscle thickness (mm) was measured for SOL and GM. No significant differences were found in SOL muscle thickness. In GM Dancers (21.7 ± 2) had significantly ($P<0.01$) thicker muscles, compared to Controls (17.7 ± 2). To evaluate the significance of thickness on stiffness or passive torque, muscle CSA would be required. Dancers may have larger GM muscle CSA resulting from daily loading. This, however

conflicts with the abovementioned training history of the Controls. Nonetheless, the dancers work 4-12 hours 5-7 days•week^{-1} and it is likely that the total loading magnitude on the MTU from strength training or stretching is substantially larger than in controls. The difference in loading magnitude may account for the differences in GM muscle thickness. While no difference was found in neither SOL nor GM pennation angle, one might have expected the dancers do display larger pennation angles due to thicker muscles and thus possible larger CSA.

7.4 Mechanical- and material properties of the MTU

As expected, the dancers displayed less (15.4±6.4 Nm) passive torque compared with controls (24.5±9.2 Nm). This underpins the results from ROM and the fact that MTU properties in Dancers and Controls are different. There were no significant differences in stiffness in the present results. After stretching interventions studies have reported increased maximal ROM unaccompanied by differences in other mechanical properties than passive torque (Magnusson et al., 1996b; Ben & Harvey, 2010). (Halbertsma et al., 1994; Halbertsma et al., 1996). With no significant differences in stiffness (Table 4), but a robust difference in ROM, the expectancy would then be differences between dancers and controls in material tendon properties (Heinemeier & Kjaer, 2011). However, neither relative nor absolute Young's modulus in GM MTU or SOL MTU was different when the two groups were compared (Table 4). Without difference in tendon CSA and stiffness, the elastic modulus in this may indicate that the there might not be intrinsic (e.g. collagen content, size of collagen fibril) differences in the MTU between Dancers and Controls. Interestingly, the Free AT in the Dancer showed significantly larger ($P<0.05$) deformation than in the Controls, while there was no difference in deformation of the GM tendon (Table 4).

In the force-elongation relationship for the Free AT (Figure 30) and GM tendon (Figure 31) calculated from 0-2500 N, there were no significant differences between the groups. However, for Free AT the elongation was different between Dancers and Controls from 100-2500N. Further, there were significant differences from 200-2500 N. The slope for both Free AT and GM tendon has is slightly right-shifted in the dancers, which is in support of present results of longer tendon lengths in Dancers. This would have implications for the intrinsic muscle properties that are responsible for force production.

Further, this implies that longer muscle fascicles are required for the muscle at optimal sarcomere length. Properties that may be influenced by this are the force-length and indeed storing and releasing elastic energy, which in turn will influence performance parameter in movements utilizing the stretch-shortening cycle.

8.0 Conclusion

The main findings are that mechanical properties of the GM tendon and the free AT were similar in both dancers and controls, but GM tendons, free AT and muscle fascicles were longer in subjects with years of stretching. The present study design does not allow for direct demonstrations of the cause and affects of the presents results. However, the volume, frequency and intensity of stretching exercise in the dancers may suggest that the differences in muscle-tendon architecture and morphology could possibly stem from their stretching training regimen.

9.0 Future perspectives

The results from this study nods in the direction of possible morphological and structural adaptations to long-term stretching exercise. Future research in a longitudinal design is needed to verify the findings of the present study. In this study the triceps surea MTU was investigated with out regard to the aponeurosis, thus care should be taken in future studies to cover as many aspects of the MTU when studying the effects of stretching. Stretching is a significant part of physical training regimens and clinical treatments, therefore it is essential to understand the mechanisms behind MTU adaptations to stretching, as well as the consequential effects on functions of the MTU.

10.0 Limitations

There are inherent limitations associated with the present study. A cross-sectional study design is inevitably impeded by training history and inter-subject variations. Without neglecting selection bias, it is important to bear in mind that the dancers' stretching training history and stretching volume is the very basis for subject recruitment and were prerequisites for the inclusion criteria. Due to the scope of a master thesis project, time did not allow for completion of all our collected data. Doubtless, the complete body of

data related to the current thesis would have helped the interpretation of the results presented here. Information regarding elongation of the different parts of the MTU during passive stretching and muscle function could have increased knowledge of MTU behavior during stretching and shed light on functional consequences for the present similarities and differences between dancers and controls.

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Table overview

Figure overview

Appendix 1: Approval from

Regional Committees for Medical and Health Research

Region: Saksbehandler:

REK sør-øst Tor Even Svanes

Jens Bojsen-Møller Norges Idrettshøyskole Sognsveien 220 0863 Oslo

Telefon:

22845521

Vår dato: Vår referanse:

02.07.2012 2012/959

Deres dato: Deres referanse:

22.05.2012 Vår referanse må oppgis ved alle henvendelser

2012/959 C Effekter av mangeårig bevegelighetstrening på struktur og funksjon i triceps surae muskel-sene komplekset .

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk i møtet 14.06.2012.

Forskningsansvarlig: Norges Idrettshøyskole **Prosjektleder:** Jens Bojsen-Møller

Prosjektomtale:

Å drive bevegelighetstrening/å ha god leddbevegelighet antas generelt å ha en rekke positive innvirkninger innen helse, rehabilitering og prestasjon. Samtidig er effektene av mangeårig bevegelighetstrening ikke tilstrekkelig dokumentert gjennom forskning. Tidligere intervensjonsstudier har vart maksimalt 24 uker,mens det antas at endringer i mekaniske egenskaper i muskel og sene krever større stimulus. Formålet med denne studien er å avdekke eventuelle forskjeller i sener, muskulatur, bindevev eller nervesystem knyttet til langvarig bevegelighetstrening blant personer som har drevet med systematisk bevegelighets trening i >10 år. Ulike analyser av vev og funksjon vil bli foretatt på ett tidspunkt for å kartlegge de overnevnte egenskapene. Sett fra et overordnet perspektiv vil studien kunne øke kunnskapen om hvilke grupper som bør eller ikke bør drive bevegelighetstrening, og hvordan treningen evt. bør foregå.

Under søknadens punkt **1.d – Andre prosjektopplysninger,** anfører søker at det her omsøkte prosjektet er relatert til studien *Mekaniske egenskaper i muskel-sene-systemet hos utøvere som har drevet mangeårig systematisk bevegelighetstrening* (referanse 2010/172). Prosjektet har som formål å få ny kunnskap om forskjellene mellom

personer med stor bevegelighet/som har trent bevegelighet over lang tid ved å sammenligne mekaniske egenskaper ved muskel-sene-systemet hos utøvere som har drevet mangeårig systematisk bevegelighetstrening (landslagsutøvere i rytmisk gymnastikk) med utøvere i idretter/mosjonsaktiviteter uten spesielt fokus på bevegelighetstrening.

Det gir begge studiene det samme utgangspunktet, fordi intervensjonsgruppen nå vil bestå av dansere fra henholdsvis Den Norske Opera og Ballett og Norges Dansehøyskole, samt en kontrollgruppe bestående av friske frivillige deltakere uten slik bakgrunn. Hensikten er igjen å sammenligne egenskaper ved muskel-sene-system.

Mekaniske egenskaper i muskel-sene-systemet hos utøvere som har drevet mangeårig systematisk bevegelighetstrening (referanse 2010/172) ble behandlet av REK sør-øst B 29.01.2010. Komiteen konkluderte den gang med at prosjektet falt utenfor helseforskningsloven, og som sådan ikke var fremleggelsespliktig for REK. Vedtaket ble begrunnet med at prosjektet hadde til hensikt å gi indikasjon om effekt av en bestemt treningsform, for å bedre prestasjon i idrett eller dagliglivet, jf. helseforskningslovens § 4 første ledd.

Besøksadresse:

Gullhaug torg 4A, Nydalen, 0484 Oslo

Telefon: 22845511 **E-post:** post@helseforskning.etikkom.no **Web:** http://helseforskning.etikkom.no/

Komiteen mener at de samme vurderingene som ble gjort tidlig i 2010, er gjeldende også for dette prosjektet. Formålet med studien er å se på effekt av bevegelighetstrening for ulike grupper av friske og (etter prosjektleders egen vurdering) sunne mennesker. På et overordnet plan kunne man sagt at studien innehar en mulig overføringsverdi til for eksempel rehabiliteringsfeltet, men det er ikke det som er den reelle hensikten med prosjektet.

Prosjektet fremstår ikke som et medisinsk eller helsefaglig forskningsprosjekt, og faller derfor utenfor komiteens mandat, jf. helseforskningslovens § 2.

Vedtak

Prosjektet er ikke fremleggelsespliktig, jf. helseforskningslovens § 10, jf. helseforskningslovens §4 annet ledd.

REK antar for øvrig at prosjektet kommer inn under de interne regler som gjelder ved forskningsansvarlig virksomhet. Søker bør derfor ta kontakt med enten forskerstøtteavdeling eller personvernombud for å avklare hvilke retningslinjer som er gjeldende.

Komiteens avgjørelse var enstemmig.

Komiteens vedtak kan påklages til Den nasjonale forskningsetiske komité for medisin og helsefag, jfr. helseforskningsloven § 10, 3 ledd og forvaltningsloven § 28. En eventuell klage sendes til REK sør-øst. Klagefristen er tre uker fra mottak av dette brevet, jfr. forvaltningsloven § 29.

Vi ber om at alle henvendelser sendes inn via vår saksportal: http://helseforskning.etikkom.no eller på e-post til: post@helseforskning.etikkom.no.

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen

Arvid Heiberg prof. dr.med leder REK sør-øst C

Kopi til: Norges Idrettshøyskole: olivier.seynnes@nih.no

Tor Even Svanes seniorrådgiver

Appendix 2:

Request to participate in study and certificate of consent

Department of Physical Performance

Request to participate in research study

"Effects of Long-Term Stretching on Structure and Function of the Triceps Surae Muscle-Tendon Complex"

Background and purpose of the research

This is a request to you regarding participation in a research study of which aim is to investigate how long-term stretching affects mechanical properties in muscles, tendons and connective tissue.
As a professional dancer on an elite level you belong to an especially interesting group of people, which can help us collect more information about how many years of stretching affects muscles, tendons and connective tissue. We hope that this study will provide us with better documentation on which changes stretching training leads to, and with that give us a better view of which groups of people may benefit from such training and which will not. This will affect both guidelines for stretching training for elite athletes, recreational athletes and patients in rehabilitation. The study and laboratory tests are approved by Regional Committees for Medical and Health Research (REK). Responsible institution is The Norwegian School of Sports Sciences.

Implications for the study

If you choose to partake in the study you will have to perform several tests regarding muscle strength, flexibility and function. Ultrasound images and video from the ankle and calf will also be collected. Please, se Attachement A for more detailed description of the tests.

Possible advantages and disadvantages with participating

By participating in the research study you will get information about your general and specific flexibility. Also, it may be possible to determine if you are genetically predisposed for obtaining large joint range of motion. Furthermore, you will receive information about the strength of your leg muscles. Information from the measurements

in the study may possibly tell what kind of effect your stretching training has had on your muscles, tendons and connective tissue. When the study is ended you will be able to compare your own measurements with average values from all the participants in this project, and you will have a good insight in how research studies are conducted. All of these factors may be useful for your further practice.

The project will require some of your personal time. All participants will, as written previously, preform a battery of tests. Everything will be completed within two (2) test days, where the first (1) day will consist of familiarization/accommodation tests as well as collecting ultrasound images. The second (2) test day is the main test day in which we will collect strength and flexibility data. Every test day can take between 30 minutes and 120 minutes. Therefore you can count on spending altogether 3 hours, divided on the different days at The Norwegian School of Sports Sciences (NIH). You will also be asked to fill out a questionnaire regarding your training status.

You will be encouraged to only move into positions that will not exceed you pain threshold, and rather be in complete control over all strength- and stretching exercises. Your execution will be carefully controlled and monitored by experienced test leaders, in order to minimize the risk of injury.

The tests made at NIH will measure your flexibility, strength and function. The strength tests shall be performed with maximal effort. To execute a maximal muscle contraction may lead to a sensation of discomfort, and may lead to some muscle soreness. Testing muscle strength can on very rare occasions
Lead to injury on muscle or tendon, *however these tests bear no higher/further risk than conducting a daily active life.* The tests procedures are commonly used in research studies and also in testing of athletes.

Ultrasonography/ultrasound examinations are conducted by holding a plastic device, called a probe, with hypo allergic gel onto the skin surface. The probe is sending sound waves and when these waves pass through various tissues, the sound is reflected back to the probe and then transformed into pictures in a PC. There is no risk or discomfort related to such examinations.

What will happen to the information we gather from your tests?

Data is collected from you and all the information gathered and registered from you shall only be used as described in the purpose of this study. All the information and test results will be treated anonymously without your name, birth number or any other directly recognizable information about you. A code will connect you to your data through a list of names that is kept behind locked doors at NIH.

Only authorized personnel involved in the project will have access to the list of names and are able to trace your special code back to you in order to guarantee your confidentiality. When the project is finalized and ended, the list of names will be deleted and your code can in no way be traced back to your name. Planned termination for the project is May 2013. All results are published anonymously.

Voluntary participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. You can at any point in withdraw your participation from the study without stating any reason for this and your withdrawal will not have any consequences for you.

If you wish to participate you may sign the Certificate of Consent below. If you decide to participate, you have the right to withdraw without further consequences

If you later decide to withdraw from the study or have further questions, please contact: Melina Meyer Magulas, telephone +47 99 29 84 97 or melinamm@student.nih.no

You will find more information about the study in Attachement A – Extensive explanation of the research study.
You will find more information about privacy and insurance in Attachement B - Privacy, economy and insurance. **You will find The Certificate of Consent after Attachement B.**

Attachment A – Extensive explanation of the research study

Criteria for participation:

- You have turned 18 years, but not 40 years
- Have no muscle- or skeletal diseases
- Have no ankle- or calf injuries the last 6 months.
    - Note: Due to nature of the tests and the fact that most dancers experience some degree of overuse injury this point is in a grey area. If you are unsure whether you should participate in the study because of this point you may inform us about your injury/discomfort and then we are able to confer with medical personnel about whether you may be tested or not.
- Have been stretching regularly the last 10 years (more than 10 minutes of stretching, 3 times PR week).

Background information for the study

Range of motion, or flexibility, is one of the factors that determines a persons ability to execute a given movement task. Stretching/flexibility training is utilized in many contexts, both in a health perspective and in sports at an elite level. Yet, there is no general agreement whether stretching training may prevent injuries or improve physical performances. This is mainly because neither the mechanisms behind change in range of motion, nor the different effects of stretching training have been verified.

There is a small number of research studies where people are exposed to stretching training over time. These studies typically have low quality, and/or have very short periods of training. This study is a an attempt to start to gather more information about possible mechanical effects of long-term stretching, in order to prepare for future studies on dancers bodies.

This study investigates how habitual stretching training affects mechanical properties in muscles, tendons and connective tissue. We hope that the study will provide us with better documentation on what types of changes long-term stretching leads to, also for what kind of populations stretching may be beneficial, and for whom it may not be beneficial. This concerns both groups of professional athletes, dancers, recreational athletes and patients.

Timeframe – what happens and when does it happen?

If you decide to participate in this study, you may respond to Melina Meyer Magulas By telephone : +47 99298497 or e-mail: melinamm@student.nih.no in order to make the first appointment.

The Informed Consent form will be signed on your first visit and before we start with any type of testing.

We would like to finish all the testing before this Christmas and based on your personal schedule and our available test time in the lab we will make the two appointments required for testing. The first test-day we will do some ultrasound examinations and familiarize you with the equipment and the protocols for strength- and flexibility testing. On the second test-day, which is the Main test, we will take you through the strength- and flexibility tests, which you were previously familiarized with.

Examinations and tests

Flexibility testing /Range of motion testing

- Passive range of motion in ankle dorsiflexion (flexed foot) will be tested in an isokinetic dynamometer. Your foot will be connected to the dynamometer, which will slowly move your ankle to the position where you feel a thorough stretch in your calf. This position will be pre-chosen by you and the dynamometer will not move beyond your chosen position.
    - You will be practicing this test with out going near your threshold for pain on the familiarization day, until you are completely comfortable with the procedure.
- Passive range of motion of the ankle will be in an area well under your threshold for pain, in an isokinetic dynamometer with ultrasound (will be explained later in this document).

Strength testing

- Before the testing starts both general and specific warm-up will be performed.
- Ankle dorsiflexion and ankle plantar flexion will be tested in the isokinetic dynamometer.
    - The tests will be conducted with isokinetic muscle action, which means that you will bend and extend your ankle in a speed that is constant no matter whom muck or how little effort you make. However, you will be asked to perform up to you max capacity.
    - Tests will be performed both concentric and eccentric.
- Ankle plantar flexion will be tested with isometric contractions in dynamometer, with use of ultrasound.
    - In this test you will be asked to gradually increase your effort, and thus your muscle force with in 6-10 seconds. The test will be conducted with isometric muscle action. This means that the machine. Testing devise keeps your ankle in the same position regardless of how great your effort is.
- You will be practicing these tests, without using your maximal force until you are completely comfortable with the procedures.

EMG

- Small electrodes that measure the activity in the muscles will be placed on the front and back of your calf. You will be wearing these electrodes while you are performing the range of motion- and strength and the functional tests.

Ultrasound

- Placing a plastic devise -a probe – is held against your skin makes ultrasound examinations. The probe sends out sound waves. When the sound waves pass through different tissues, some of the sound will be reflected back to the probe and are then turned into images of film in a PC. There is absolutely no discomfort or risk connected to such ultrasound examinations.
- Ultrasound is utilized as a tool in some of the range of motion- and strength tests described above.
- In addition ultrasound images will be taken of gastrocnemius medialis, soleus and the Achilles tendon while you perform a functional ankle movement test in the also isokinetic dynamometer.

Anthropometric measurements

- Measurements of height, weight, leg length and calf length.

Questionnaire

- Dominant leg – which leg do you prefer to kick up in a grand battement?
- Training background
- Current training habits
- Current/pervious injuries

Possible advantages to participating

By participating in this study you will get information about the strength and flexibility in tour legs. You will get practical experience with several scientific ways of testing flexibility and muscle function, and you will also get a proper insight in how a research study is conducted. This may all be useful for your further practise.
You get to take part in an innovative study, which is the first of its kind. Thus, you are a contributing to increase the knowledge to a research field which will affect directly affect your profession with thought to guidelines for stretching and flexibility training and to injury prevention for dancers.

Possible discomfort

The project will demand some of your personal time. As before mentioned, all participants will perform a battery of tests. There are two different test days: Day 1 consists of familiarization and accommodation to the test procedures. Day 2 is used for testing and data collecting. Procedures on each of the two days may take 30-120 minutes. Therefore, you may make altogether 3 hours of your time available to the procedures at NIH. The 3 hours are divided by two days. We will also ask you to fill out a questionnaire regarding your training status and training history. This questionnaire is anonymous.

To stretch might be viewed as uncomfortable and you will be encouraged to only comply with positions that are within you threshold of pain. At all times you will have full control over the stretching intensity. This is a gentle form of training and you will receive sufficient training in the procedures.

The testing conducted at NIG will measure four flexibility, strength and function. The strength tests must me conducted with maximal effort, which may be associated with some level of discomfort, and might induce delayed onset muscle soreness. Strength testing might lead to some types of injuries on muscles or tendons, however the risk of this happening during testing is no greater than leading an active life.

Ultrasound of the muscle-tendon system bears no discomfort or risk

Economy
Subjects with a very long travel route may be reimbursed for the cost of their trip to NIH after an individual agreement.

You have the right to information
All tests in the project have been thoroughly tried and tested before the start of the whole project. If this pervious testing has lead to information that you ought to know about, we will inform you about this.

Attachment B - Privacy, economy and insurance

Privacy

Information about you that will be registered is: Name, age, training history and results from the testing described in Chapter A.
All the information about you will only be used as describes in line with the study. The information will be confidential, and will be treated with out your name, birth number or other information that could be traced back to you. A code will connect you to your information though a name list, safely stored.

Only authorized personnel have access to the name list and are able to trace your code back to you. When the results from the project are processed and the project closed, the name list will be destroyed, and it will not be possible to trace the information obtained back to you. The completion of the present project is anticipated to be en of May 2013.

It will not be possible to identify you when the study is published.

At The Norwegian School of Sports Sciences the administrating director is responsible for data processing.

Sharing of information

Data collected from you in relation to this study, and also information about you cannot be given to other research institutions, but will be kept at NIH.

The right to information and deleting of information and deleting of test data

Upon agreeing to participate in this study, you have the right to information regarding what data is registered about you. You further have the right to correct mistakes in the information gathered about you, in the eventuality that this should happen. If you decide to withdraw from the study, you have the right to demand that collected data and information gathered about you is deleted, unless the information is already part of analyses or used in scientific publications.

Economy and the role of The Norwegian School of Sports

The study is a Master of Sports Sciences project, financed by funds from the Norwegian School of Sports Sciences. Any conflicts interests, ethical or practical challenges, do not compromise this funding.

Insurance

Participants in this study are insured through *Norges Idrettshøgskoles næringslivsforsikring hos Gjensidige.*

Information regarding the outcome of the study

As participant in the project you have the right to know your own results, and also information regarding the total study results. This information will be sent to the participants when the project is finished. You can also receive this information by contacting: melinamm@student.nih.no

Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have been asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study

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(Signature by participant, date)

Deputy gives consent when entitled, instead of or in addition to the participant

---

(Signature by closely related deputy, date)

I have accurately provided the information sheet for the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

(Signature, role in the study, date)

Appendix 3: Permission to use figure

E-mail correspondence:

Re: Permission to use figure from paper

Katja Heinemeier 15.04.2014

Til: melina magulas

Fra: **katja heinemeier** (katjaheinemeier@gmail.com)
Sendt: 15. april 2014 12:56:37
Til: melina magulas (melina_magulas@hotmail.com)

Hi Melina,

Of course you are more than welcome. Thanks for asking though.

Send my regards to Jens and Olivier.

Best wishes, Katja
--
Katja Heinemeier, PhD
Institute of Sports Medicine - www.ISMC.dk
Bispebjerg Hospital
Building 8, 1. floor
Bispebjerg Bakke 23
2400 Copenhagen NV
Denmark

Tlf: 0045 2855 6602
Fax: 0045 3531 2733
11.

Katja Heinemeier

Vis kontakt

2014-04-15 12:14 GMT+02:00 melina magulas <melina_magulas@hotmail.com>:
Hi Katja,

I am a Master student at The Norwegian School of Sports Sciences currently working on a thesis with the title: *Effects of Long-Term Stretching on Structure and Function of the Triceps Surae MTU in Elite professoinal Dallet Dancers.* I am supervised by Jens Bojsen-Møller and Olivier Seynnes.

I am writing to you because I would like to use a figure from your paper in a theory chapter of my thesis. The paper is: In vivo Investigation of Tendon Responses to Mechanical Loading and it is figure 4 I am asking your permission to use.

Thank you for great contributions to my understanding of tendon properties through your research.

Best regards,

Melina Meyer Magulas

Appendix 4: Questionnaire

QUESTIONNAIRE, PROFESSIONAL DANCER

1	Research subject no:	(to be filled in by the researchers)
2	Date of birth:	
3	Which sports or types of exercise have you practised the last 3 years, except dance training?	
4	Which exercises could you already do when you started practising dance?	
4a	Splits on the floor, right leg	○ Yes ○ No ○ Don't know
4b	Splits on the floor, left leg	○ Yes ○ No ○ Don't know
4c	Side splits on the floor	○ Yes ○ No ○ Don't know
4d	Bend forward with straight knees, touch the floor	○ Yes ○ No ○ Don't know
5	How old were you when you started practising dance?	
6	How old were you when you started your training in classical ballet?	
7a	Women: How old were you when you started training on pointe?	
7b	How many hours do you practice on pointe per week, season 2011/2012?	
8	How many hours do you exercise/rehearse each week, in 2011/2012?	
9	How many hours do you stretch each week, season 2011/2012?	
10	Which is your best leg for doing splits?	○ Right ○ Left ○ No difference
11	Which leg do you prefer to take off from in leaps/jump	○ Right ○ Left ○ No difference
12	If you have had any injuries to your legs during the last 3 years, please describe them (type of injury, when, and was the injury treated by health personell):	