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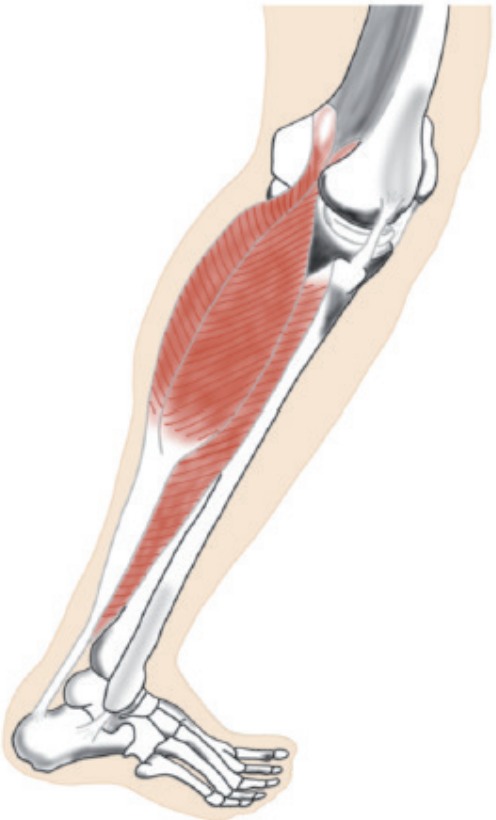
Lauri Stenroth

Structure and Function of Human triceps surae Muscle and Tendon in Aging



JYVÄSKYLÄN YLIOPISTO

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Esitetään Jyväskylän yliopiston liikuntatieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston vanhassa juhlasalissa S212
syyskuun 2. päivänä 2016 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Sport and Health Sciences of the University of Jyväskylä,
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2016

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JYVÄSKYLÄ 2016

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Pekka Olsbo
Publishing Unit, University Library of Jyväskylä

URN:ISBN:978-951-39-6715-4
ISBN 978-951-39-6715-4 (PDF)

ISBN 978-951-39-6714-7 (nid.)
ISSN 0356-1070

Jyväskylä University Printing House, Jyväskylä 2016

ABSTRACT

Stenroth, Lauri
Structure and function of human triceps surae muscle and tendon in aging
Jyväskylä: University of Jyväskylä, 2016, 87 p.
(Studies in Sport, Physical Education and Health
ISSN 0356-1070; 242)
ISBN 978-951-39-6714-7 (nid.)
ISBN 978-951-39-6715-4 (PDF)

Aging is associated with loss of muscle mass leading to impairments in muscle function, such as loss of muscle strength or power. These impairments may eventually lead to physical disabilities. Although loss of muscle mass has a major role in age-related loss of muscle function, there is a disproportionate loss of muscle strength and power with aging suggesting that other factors are also involved. These other factors may include age-related modifications in muscle architecture and tendon mechanical properties, factors that have a significant effect on muscle function. Currently there is no consensus about age-related changes in muscle architecture or tendon mechanical properties, or their role in age-related impairments of physical function. Therefore, the aim of this thesis was to examine associations between aging and muscle architecture, tendon mechanical properties and muscle-tendon function during walking in the human triceps surae muscle by comparing healthy young and older adults. Associations between tests of physical function and triceps surae muscle-tendon properties were also investigated in older adults. The results suggested only modest effects of aging on triceps surae muscle architecture and Achilles tendon mechanical properties. However, inter-individual differences in triceps surae muscle architecture and Achilles tendon stiffness explained variance in 6-minute walk test and in timed "up-and-go" –test in older adults. Muscle-tendon function was found to be dependent on walking speed in older adults, with no significant differences between young and older adults when compared at matched walking speed. These results suggest that the commonly observed age-related slowing of preferred walking speed may represent an attempt to compensate for loss of muscle strength in triceps surae muscle. In conclusion, the results of the current thesis suggest that aging may be related to changes in triceps surae muscle architecture and Achilles tendon mechanical properties. However, these changes seem to be part of an adaptation process that helps to maintain muscle function despite declines in muscle mass and strength with aging.

Keywords: triceps surae, Achilles tendon, muscle architecture, stiffness, aging

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ACKNOWLEDGEMENTS

I began my studies in sports science majoring in biomechanics without really knowing what biomechanics is. However, it became clear to me at the very beginning of my studies that I had chosen the right discipline. Lectures given by Professor Taija Finni were the ones that got me really excited about biomechanics. She has continued to be a source of inspiration throughout my studies ever since.

I am most grateful for my supervisors: Professors Taija Finni and Sarianna Sipilä and Docent Neil Cronin. One could not have hoped for better supervisors; Taija's and Neil's expertise in muscle-tendon biomechanics and Sarianna's vast experience and expertise in exercise gerontology were an unbeatable combination. With their guidance they ensured my growth from student to researcher.

I started to work with tendons when I had a chance to work in Dr. Jussi Peltonen's project for my bachelor's thesis. That was the starting point of our collaboration and friendship. I have learned most of my skills in measurement techniques and analysis from Jussi. The numerous fruitful discussions led to great ideas and better understanding of the matters discussed. I would not be here today without your help.

I want to thank all the students and staff both at the Department of Biology of Physical Activity and at the Gerontology Research Center at the Department of Health Sciences. It has been a privilege to work with and deepen my understanding of the human locomotor system with you. The atmosphere that such intelligent people create has been truly inspiring and you have made the journey pleasant and fulfilling. I particularly want to thank Arto Pesola who has walked the same path with me since the start of my studies.

I thank the technical staff of the department, Markku Ruuskanen and Sirpa Roivas, who keep the labs running and who have been miracle makers if some device happened to malfunction. I also want to take this opportunity to thank the department's secretaries, Katja Pylkkänen and Minna Herpola, who keep the department running and help with various daily tasks.

I also want to thank my high jump coach, Juha Isolehto. He has been an important person during my years in Jyväskylä. He inspired me to study sport science and biomechanics.

My parents, Eija and Markku, planted the seed of my scientific career with their respect for education and scholarship. They gave me a safe and secure home to grow up in. I am also lucky to have an older brother, Ilari, to look up to. I am thankful to Teija and Ari, my mother and father in law, for their support and encouragement during the process. I am fortunate to have such a loving family.

Financial support for the preparation of this theses and associated congress trips have been provided by The European Union, The Finnish Cultural Foundation, Emil Aaltonen Foundation and Jyväskylän yliopiston tieteentekijät RY. Also the Department of Biology of Physical Activity and the Department of Health Sciences have significantly contributed to this thesis by providing facilities to conduct the studies. None of the studies would have been possible with-

out the subjects. I am thankful for your participation. I also want to thank all of my coauthors of the original publications for your help.

Finally, I am most grateful to my wife Elisa. You have supported me daily throughout my studies and thesis writing. You have always managed to encourage me and push me forward. You have shared the load when it has been too heavy to be carried alone. Most importantly, you have reminded me that there is still life outside science. I look forward to our future adventures.

Jyväskylä 24.4.2016
Lauri Stenroth

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on four original papers, which are referred to by the following roman numerals:

I Stenroth L, Peltonen J, Cronin NJ, Sipilä S & Finni T. 2012. Age-related differences in Achilles tendon properties and triceps surae muscle architecture in vivo. Journal of Applied Physiology 113(10), 1537-1544.

II Stenroth L, Sillanpää E, McPhee JS, Narici MV, Gapaeyeva H, Pääsuke M, Barnouin Y, Hogrel J-Y, Butler-Browne G, Biljsma A, Meskers CGM, Maier AB, Finni T & Sipilä S. 2015. Plantarflexor muscle-tendon properties are associated with mobility in healthy older adults. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 70(8), 996-1002.

III Stenroth L, Cronin NJ, Peltonen J, Korhonen MT, Sipilä S & Finni T. 2015. Triceps surae muscle-tendon properties in older endurance- and sprint-trained athletes. Journal of Applied Physiology 120, 63-69.

IV Stenroth L, Sipilä S, Finni T, Cronin NJ. Slower walking speed in older adults improves triceps surae force generation ability. Medicine & Science in Sports & Exercise, doi: 10.1249/MSS.0000000000001065.

ABBREVIATIONS

CSA	Cross-sectional area
EMG	Electromyography
LG	Lateral gastrocnemius
MG	Medial gastrocnemius
MTJ	Muscle-tendon junction
MTU	Muscle-tendon unit
MVIC	Maximal voluntary isometric contraction
TT	Tendinous tissue
TUG	Timed “up and go” –test
6MWT	6-minute walk test

CONTENTS

1 INTRODUCTION

In a policy framework for active aging, the World Health Organization has stated that actions promoting active and healthy aging should aim to provide older adults continued possibilities for health, participation in society and security (WHO 2002). Important aspects of active aging, as described by The World Health Organization, are independence and participation in the community. It is well documented that aging is associated with loss of skeletal muscle mass, (Mitchell et al. 2012). Skeletal muscles (hereafter simply called muscles) are the actuators that power movement, and hence age-related loss of muscle mass may eventually lead to physical disabilities and loss of functional independence (Jette et al. 1998) compromising active aging.

Understanding the causes of impairments in muscle function with aging, e.g. loss of muscle strength and power, has paramount importance for the attempts to support healthy and active aging. Impairments in muscle function are not simply due to loss of muscle mass. Longitudinal observations from older adults show that age-related loss of muscle strength exceeds the loss of muscle mass by approximately three fold and even maintaining muscle mass does not prevent the loss of muscle strength (Goodpaster et al. 2006, Delmonico et al. 2009). These findings highlight the fact that age-related loss of muscle function is not exclusively the result of loss of muscle mass, but is a multifactorial process including changes in the nervous and muscular systems (Clark & Manini 2012).

Muscle architecture and tendon mechanical properties are major determinants of muscle mechanical function (Zajac 1989). Previous studies have identified age-related differences in muscle architecture and tendon mechanical properties between young and older adults. Hence, these possible age-related changes may contribute to loss of muscle function and the disproportionate decline in muscle function compared to muscle mass.

Ankle plantarflexor muscles have an important role in human locomotion. In walking, they provide propulsion in late stance, control of body rotations and support for body mass (Sutherland et al. 1980, McGowan et al. 2009, Francis et al. 2013, Lenhart et al. 2014, Honeine et al. 2013). The most profound age-related physical limitation in walking is the loss of ankle joint power in late

stance (Kerrigan et al. 1998, DeVita & Hortobagyi 2000, Kulmala et al. 2014). Similarly, computer simulations suggest that walking ability is most sensitive to impairments in muscle strength of the plantarflexors (van der Krogt et al. 2012). Adding to the importance of plantarflexors is the fact this muscle group has the largest physiological cross-sectional area of all lower limb muscle groups (Ward et al. 2009). It has been suggested that plantarflexors are utilized at their maximal capacity during walking in older adults (Beijersbergen et al. 2013). In addition, plantarflexors may be more susceptible to age-related loss of muscle strength than other muscle groups (Christ et al. 1992).

Understanding the mechanisms of age-related loss of muscle function is a key to understanding how to maintain physical function in older age. Yet, limited knowledge exists regarding the effects of age-related changes in muscle architecture and tendon mechanical properties on the age-related loss of muscle function. Firstly, age-related differences in muscle architecture and tendon mechanical properties are not well defined. Secondly, only a limited number of studies have tried to address the functional relevance of these changes. Therefore, the current thesis aims to improve knowledge of these important issues. The studies concentrate on the main ankle plantarflexor, the triceps surae muscle group, because of its functionally important role in locomotion.

2 LITERATURE REVIEW

2.1 Muscle-tendon unit structure

Muscle-tendon unit (MTU, Fig. 1) is defined as the muscle cells (fibers) and connective tissue structures extending from the proximal to distal bone-tendon junction. The MTU can be considered as the functional unit responsible for generation of movement of joints.

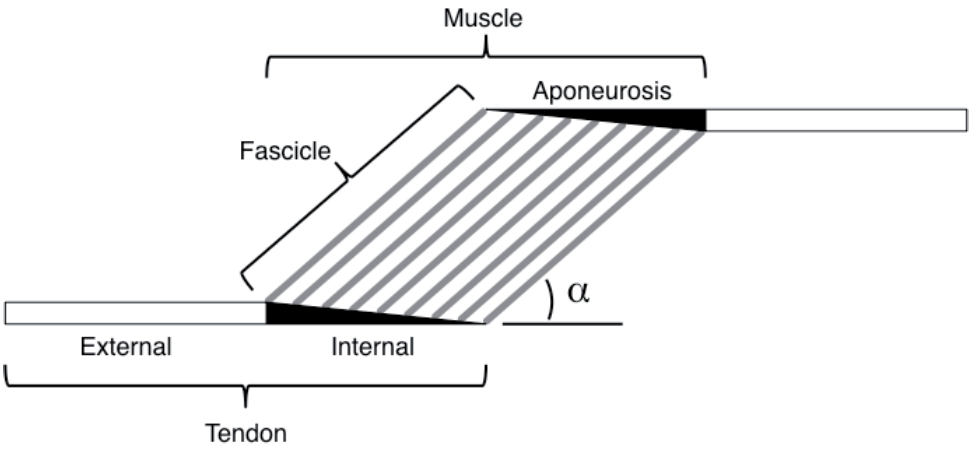

FIGURE 1 Parallelogram model of a muscle-tendon unit with pennate muscle fiber arrangement. The muscle-tendon unit is comprised of muscle fibers and connective tissue structures extending from proximal to distal bone-tendon junctions. Muscle fibers/fascicles (gray lines) run between distal and proximal tendons. Tendon can be divided into external and internal parts. The internal part is called aponeurosis. Muscle fascicles are aligned at an angle α (pennation angle) in relation to the longitudinal axis of the tendons. Figure is redrawn after Zajac (1989).

A simplified model of a MTU is presented in figure 1. Here, muscle (also called muscle belly) refers to the part of the MTU extending from distal to proximal muscle-tendon junction (MTJ). At both ends, muscle is attached to tendons that connect MTU to bones. The external part of the tendon is also called free or outer tendon. Tendons may continue inside the muscle as internal tendons called aponeuroses, which serve as attachment surfaces for muscle fascicles (bundles of muscle fibers).

2.1.1 Muscle architecture

Muscle architecture refers to the geometrical arrangement of muscle fibers within a muscle (Chow et al. 2000). Muscle architecture can be defined using variables such as fascicle length, pennation angle, anatomical and physiological cross-sectional area (PCSA) and muscle length.

Muscle fascicles run between two connective tissue surfaces, aponeuroses. Although muscle fascicle and fiber are often synonymously used when referring to muscle architecture, it should be noted that muscle fibers do not necessary extend the whole length of the muscle fascicle (Ounjian et al. 1991). However, the assumption that fascicle length also reflects fiber length has been made when estimating the number of sarcomeres in series (Cutts 1988). Furthermore, from a functional perspective, fibers connected in series to form a fascicle can be assumed to act mechanically like a single long muscle fiber (Bodine et al. 1982). Thus, measurements of muscle fascicle length and orientation can be used to reflect the anatomical arrangement of muscle fibers.

Pennation (or pinnation) angle has been defined as the angle of muscle fibers relative to the tendon of insertion (Wickiewicz et al. 1983). When using ultrasound imaging for measurements of muscle architecture, pennation angle is typically defined as the angle of the muscle fascicle relative to the aponeurosis of insertion or origin (Fig. 2).

Muscle cross-sectional area (CSA) can be measured perpendicular to muscle's longitudinal axis (anatomical CSA) or perpendicular to muscle fibers (physiological CSA). PCSA reflects the number of sarcomeres in parallel and consequently is directly proportional to a muscle's force generation capacity (Wilson & Lichtwark 2011). PCSA can be estimated using the equation PCSA=muscle volume/fascicle length. This estimates the number of sarcomeres in parallel but does not take into account the effect that pennation angle has on muscle force transmitted in the direction of the tendon. Thus, the equation PCSA=muscle volume/fascicle length*cos(pennation angle) can be used to estimate muscle force generation capacity (Narici et al. 2016).

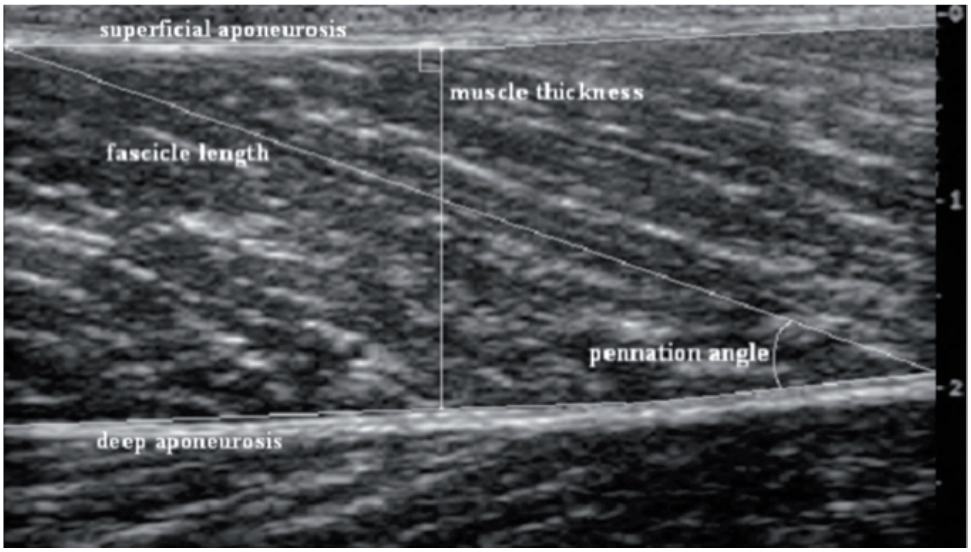

FIGURE 2 An example of a B-mode ultrasound image of medial gastrocnemius muscle with measurement of fascicle length, pennation angle and muscle thickness shown. Image reproduced with permission from Oxford University Press (Atkinson et al. 2010).

Muscles can be grouped based on their architectural design (Fig. 3). In fusiform muscle the muscle fibers run parallel to the muscle's longitudinal axis. In pennate or fan-shaped muscle, fascicles run at an angle relative to the muscle's line of action. Pennate muscles can be further separated into uni-, di- or multipennate muscles (Lieber & Ward 2011).

There are significant architectural variations between different human muscles. Within lower limbs, there can be 10 fold differences in muscle fascicle lengths ranging from under 4 cm (tibialis posterior) to over 40 cm (sartorius), and 20 fold differences in pennation angle ranging from almost no pennation (sartorius) to about 30 degrees (vastus medialis). Differences in muscle design are highlighted by the ratio between muscle fascicle to muscle length, which reflects the muscle's excursion range. These values range from 0.11 for soleus to 0.9 for sartorius. Despite the variation in the muscle architectural variables between different muscles, the same muscle across different individuals shows only small variation in the architectural variables (Ward et al. 2009). The functional significance of muscle architectural features is discussing in more detail in chapter 2.2.1.3.

Before the development of medical imaging, muscle architectural measurements from humans could only be made from cadavers. Nowadays the most common method for muscle architectural studies is ultrasound imaging. Architectural measurements can also be made using magnetic resonance imaging with diffusion tensor imaging, but this method is more expensive and time consuming, although it does allow for three dimensional determination of muscle architecture (Schenk et al. 2013, Sinha et al. 2014, Bolsterlee et al. 2015). With

ultrasound imaging it is possible to noninvasively visualize muscle architectural features and make measurements (Cronin & Lichtwark 2013). However, ultrasound imaging is not capable of visualizing individual muscle fibers but rather bundles of fibers called fascicles. Muscle fascicles are surrounded by extracellular connective tissue called perimysium (Kjaer 2004), and it is the connective tissue structures that are readily visible as white striations in a B-mode ultrasound image. Muscle volume needed for in vivo calculations of PCSA, can be obtained using magnetic resonance imaging (Morse et al. 2005c) or a series of spatially oriented ultrasound images (Barber et al. 2009), and fascicle length can be obtained using ultrasound imaging (Morse et al. 2005c).

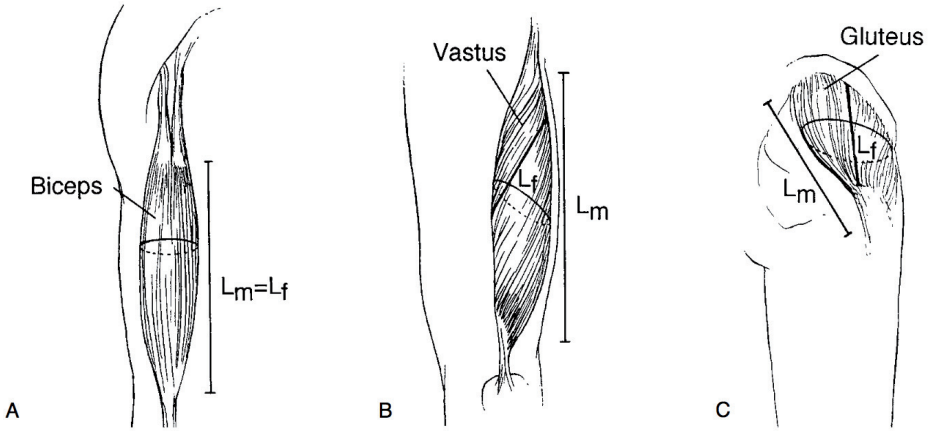

FIGURE 3 Examples of different muscle architectural types. From left to right the images represent fusiform (a), pennate (b) and multi-pennate (c) muscles. In fusiform muscle the muscle fibers are organized parallel to the muscle's line of action. In pennate muscle the fibers are organized at an angle relative to the muscle's line of action, and in multi-pennate muscle the angle of the fibers in relation to the muscle's line of action varies in different parts of the muscle. It should be noted that in fusiform muscle, muscle fascicle length is almost the same as muscle length and anatomical cross-sectional area coincide with physiological cross-sectional area. Image reproduced with permission from Wolters Kluwer Health, Inc. (Lieber & Friden 2001).

2.1.2 Tendon and aponeurosis

Tendon is a collagen rich connective tissue providing a mechanical link between muscle and bone. Tendon may also continue inside the muscle as a sheet-like extension called aponeurosis. Aponeurosis provides an attachment surface for muscle fibers through which they can exert force on to the tendon. Structurally, aponeurosis is an extension of the external tendon inside the muscle but mechanical properties may differ between external tendon and aponeurosis (Finni et al. 2003; Magnusson et al. 2003).

Morphologically, tendons varies considerably in size and shape from flattened to rounded (O'Brien 1997). Despite the variation in overall structure, internal structure is highly conserved across tendons. The internal structure is dictated by hierarchical organization of the extracellular collagen matrix (Fig. 4).

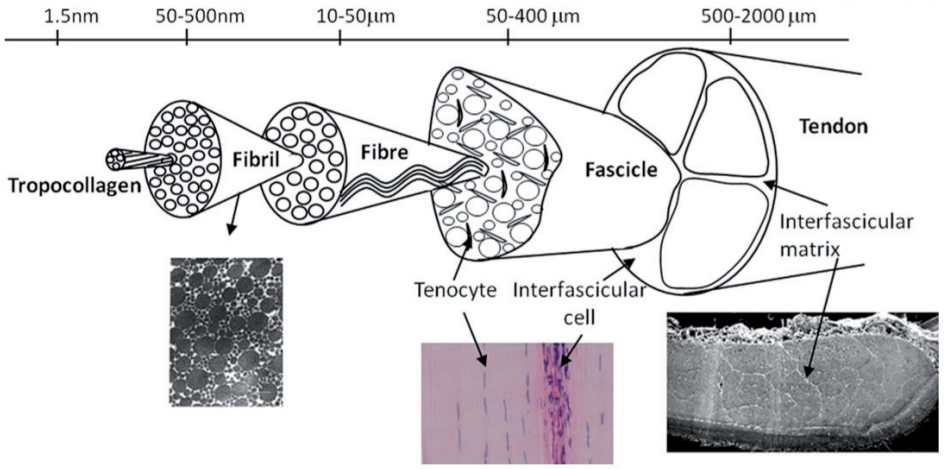

FIGURE 4 Tendon hierarchical structure. Tendon extracellular collagen matrix is hierarchically formed from tropocollagen molecules. Fibril and fascicle packing is shown in the transverse plane and the middle inset shows a longitudinal histological section with tendon cell populations. Image reproduced with permission from John Wiley and Sons (Screen et al. 2015).

Collagen molecules (tropocollagen) form fibrils and are bound together by covalent cross-links (Eyre & Wu 2005). Collagen fibrils are the primary loadbearing component in tendon (Screen et al. 2015). Fibrils group together to form fibers, which are grouped together to form fascicles and finally the whole tendon. Collagen fibers and fascicles are surrounded by a connective tissue sheet called endotenon and the whole tendon is surrounded by epitenon (Galloway et al. 2013).

Tendons are relatively hypocellular with one of the largest matrix to cell ratios of any human tissue (Screen et al. 2015). About 55-70% of tendon weight is water. Collagen makes up about 60-85% of tendon's dry mass from which type I collagen covers about 95% (Kjaer 2004). Small levels of collagen types III, V, XI, XII and XIV are also found (Thorpe et al. 2013). The rest of the tendon dry mass includes elastin, proteoglycans and associated glycosaminoglycans and other proteins but their relative contribution to tendon composition is not well defined (Kirkendall & Garrett 1997, Kjaer 2004, Screen et al. 2015). The role of these matrix components in tendon mechanical and biological properties is largely unknown, but proteoglycans have a role at least in water attraction and tendon resistance to compression (Yoon & Halper 2005). Specific compositional data on human Achilles tendon is currently lacking (Freedman et al. 2014).

2.1.3 Triceps surae muscle

Triceps surae, or calf muscle, is a muscle group situated at the posterior aspect of the lower leg. It is comprised of soleus and the medial and lateral heads of gastrocnemius (Fig. 5). All three muscles attach distally to a common tendon, the Achilles tendon, but have separate proximal origins. Soleus lies under gastrocnemius and originates from the head and upper third of the dorsal surface of the fibula. It also extends more distally compared to gastrocnemius muscles. Medial and lateral heads of gastrocnemius originate from the superior surfaces of medial and lateral femoral condyles, respectively. The main function of triceps surae is ankle plantarflexion. As the gastrocnemius muscles cross the knee joint, they also assist in knee flexion.

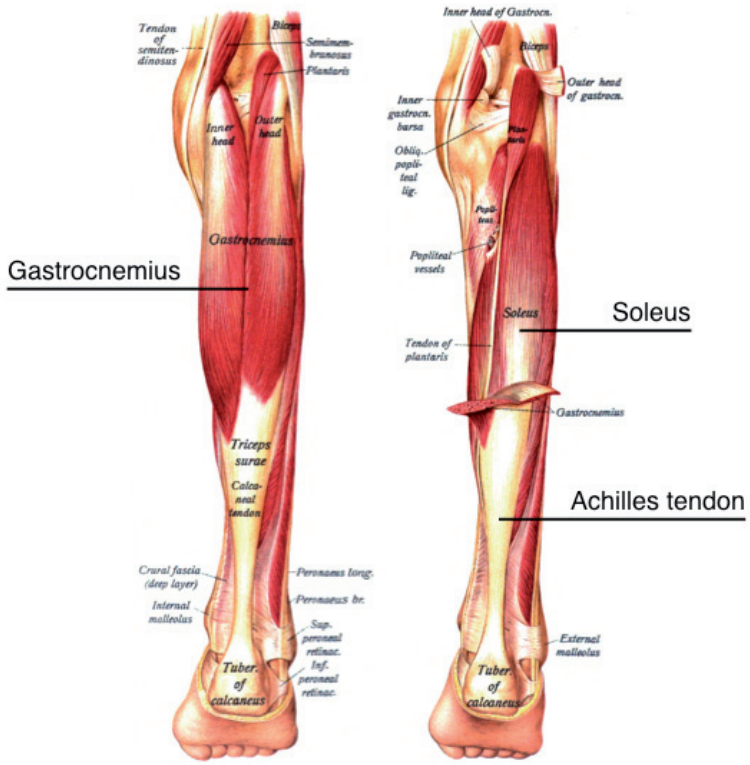

FIGURE 5 Posterior view of the right lower leg showing triceps surae muscle group and Achilles tendon. On the right side, medial and lateral heads of gastrocnemius muscle have been cut and pealed down to show the underlying soleus. Achilles tendon extends from the muscle-tendon junction to its insertion on the calcaneus (heel bone). Figure adapted from Sobotta's Atlas and Textbook of Human Anatomy 1909 (public domain image).

Gastrocnemius and soleus are pennate muscles. Both medial and lateral gastrocnemii are unipennate meaning that they have one muscle compartment in which the muscle fiber alignment is relatively uniform. The structure of soleus is more complex, with individual variations reported (Hodgson et al. 2006). It

has two compartments: anterior and posterior. The posterior compartment is unipennate while the anterior compartment has a bipennate structure (Chow et al. 2000).

Muscle fascicle lengths in triceps surae muscle are relatively short compared to muscle length due to a pennate muscle fiber configuration. Muscle fascicle lengths have been reported to be on average 19%, 27% and 11% of the muscle belly length for medial and lateral gastrocnemius and soleus, respectively (Ward et al. 2009). Thus, the muscle architecture of triceps surae muscle favors large force generation capacity as opposed to large muscle excursion or fast shortening velocity (Lieber & Bodine-Fowler 1993).

Force generation capacity of a muscle can be estimated from its PCSA. Soleus has the largest PCSA among triceps surae muscles and also among all lower limb muscles. Thus based on PCSA, soleus is the strongest lower limb muscle. The PSCA of soleus is about three times greater than that of medial gastrocnemius (MG) and five times greater than that of lateral gastrocnemius (LG) (Ward et al. 2009).

2.1.4 The Achilles tendon

The Achilles tendon is the largest and strongest of human tendons (Harris & Peduto 2006). At one end it is connected to triceps surae muscle and at the other end to calcaneus. The free tendon is about 5-6 cm long and has a diameter of about 5 mm (Fornage 1986).

The Achilles tendon is formed from tendon fascicles (bundles of fibers) arising from each head of triceps surae and enclosed in a connective tissue sheet. These bundles of fascicles twist along their course from muscle to calcaneus insertion. In general, fascicles arising from soleus insert on the medial and anterior side of calcaneus, fascicles from MG on the posterior and lateral side and from LG on the lateral to anterior side but, individual variation exists (Edama et al. 2015, Szaro et al. 2009). It has been proposed that the twisting of the fascicles may have a functional role, for example allowing high strains and energy storage (Bojsen-Møller & Magnusson 2015).

The Achilles tendon can sustain high forces. Forces up to 9 kN or 12.5 times body weight have been measured in the Achilles tendon using a buckle transducer inserted to the tendon (Komi 1990). The Achilles tendon can also sustain high strains. Average peak strain during hopping has been measured to be about 7-8% (Hoffrén et al. 2012, Lichtwark & Wilson 2005a). These qualities give the Achilles tendon a high capacity for elastic energy storage. Hence, the Achilles tendon has a significant role in energy saving during locomotion. For example in hopping, the energy stored in the Achilles tendon during ground contact provides on average 16% of the total mechanical energy of the hop (Lichtwark & Wilson 2005a). Energy saving mechanisms of tendons are discussed in more detail in chapter 2.2.3.

2.2 Muscle-tendon unit function

MTU mechanical function is determined by intrinsic properties of its muscle fibers, muscle architecture, tendon mechanical properties and moment arm (Gans & Bock 1965, Lieber & Ward 2011, Zajac 1992). Selected aspects of MTU mechanical function that are important in the context of the current thesis are reviewed in this chapter. First, fundamental mechanical properties of different components of MTU (i.e. muscle and tendon) are reviewed with discussion of the functional importance of muscle architecture. Whole MTU function is then considered, and the process of interaction between muscle and tendon is explained.

2.2.1 Muscle mechanics

Muscle function is greatly dependent on the mechanical properties of its muscle fibers. Two fundamental properties of muscle fibers determine their mechanical behavior, force-length and force-velocity relationships. The general formulation of these properties is highly conserved irrespective of muscle fiber type. In addition to length and velocity, muscle force generation is dependent on history of muscle contractions and mode of previous muscle actions but these properties of muscle are beyond the scope of this thesis. Interested readers are referred to reviews by Rassier and Herzog (2004) and Hodgson et al. (2005).

2.2.1.1 Force-length relationship

Muscle force generation capacity depends on its length. This can be observed at sarcomere, fiber, MTU and at joint level (Rassier et al. 1999). The force-length dependency at any level arises from the force-length dependency of sarcomeres. In 1966, Gordon et al. was able to show with unforeseen detail that muscle fiber force varied according to sarcomere length (Gordon et al. 1966). Moreover, the observation was in agreement with the sliding filament theory. Based on this theory, muscle force generation capacity is dependent on the overlap between myosin and actin filaments, which consequently determine the number of myosin cross-bridges that can be formed between the filaments. Overlap between myosin and actin filaments is determined by sarcomere length, and hence muscle force generation capacity varies according to muscle length (Huxley & Niedergerke 1954, Huxley & Hanson 1954).

The sarcomere length that produces the greatest force in maximally activated muscle is called the optimal length. There is no single optimal sarcomere length but rather a small range of sarcomere lengths that form the highest values in the force-length relationship, called the plateau region. In human muscles the plateau region is estimated to be between sarcomere lengths of 2.64-2.81 μm (Rassier et al. 1999). Experimental findings support this estimation with optimal lengths found between 2.54-2.78 μm and a mean optimal length of 2.66 μm (Gollapudi & Lin 2009). Force generation capacity is reduced at both shorter and longer sarcomere lengths from the plateau forming the classical bell or in-

verted parabola -shaped force-length relationship (Gordon et al. 1966). Active force generation by sarcomeres or muscle fibers in human muscle is possible within lengths ranging from approximately 0.5 to 1.5 times the optimal length (Zajac 1989, Hill 1953).

It should be noted that the inverted parabola represents active force generation by the muscle. The total force of the muscle is a result of active and passive forces. Passive forces arise as a result of stretch of muscle's internal elastic components. To derive the classical force-length relationship one must subtract passive force from the total force (Fig. 6). In chemically skinned human muscles, the average length at which passive force generation emerges (i.e. slack length) is around 2.22 μm (Gollapudi & Lin 2009) but in whole muscles the slack length probably varies between muscles (Rassier et al. 1999).

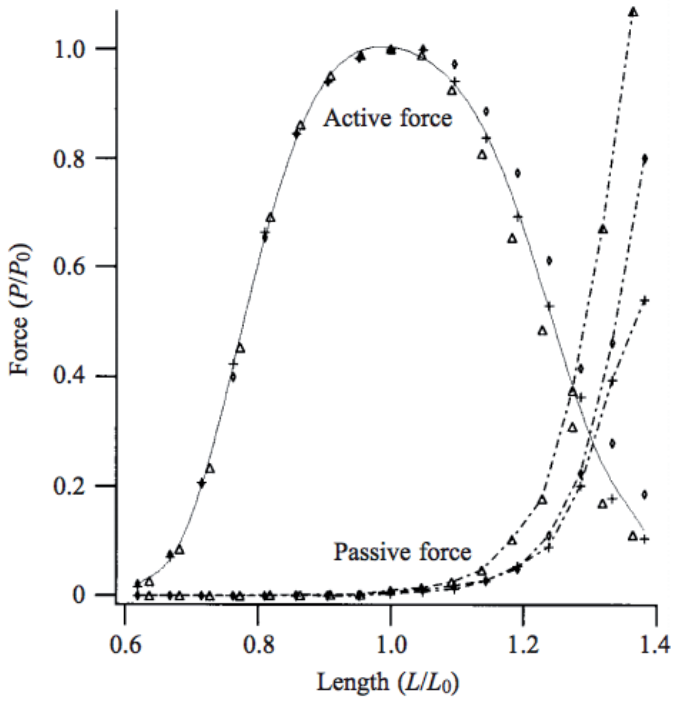

FIGURE 6 Force-length relationships of three mouse soleus muscles. The curves represent steady-state force at different muscle-tendon unit lengths normalized to length at which peak active force occurs. Total force measured (not shown) is the sum of force of the passively stretched muscle-tendon unit and the extra force generated by maximally activated muscle. Picture reproduced with permission from Company of Biologists LTD. (Askew & Marsh 1998).

Sarcomere lengths can be measured in vivo using laser diffraction (Lieber et al. 1994) or optical microendoscopy (Llewellyn et al. 2008) methods, and thus it is possible to determine force-length relationships at sarcomere level in vivo. However, both of the aforementioned methods are invasive, which has limited their use in human studies, and no data exist on human triceps surae sarcomere

operating range on the sarcomere force-length relationship. Estimations have been made based on muscle architectural data (Cutts 1988), joint torque measurements (Herzog et al. 1991, Winter & Challis 2010), joint torque measurements combined with ultrasound imaging (Rubenson et al. 2012; Maganaris 2003), ultrasound imaging combined with cadaver data (Fukunaga et al. 2001) and computer simulations (Arnold & Delp 2011). The results of these studies are quite uniform and suggest that human triceps surae muscles operate on the ascending and plateau regions of the force-length relationship.

2.2.1.2 Force-velocity relationship

Muscle force generation capacity depends also on the velocity of contraction, with lower forces generated as shortening velocity increases (Fenn & Marsh 1935). The force-velocity relationship of a shortening muscle was first described by Hill in 1938 (Hill 1938). The general shape of the force-velocity relationship for muscle shortening is presented in figure 7 and can be approximated by a hyperbolic function. Similarly to the force-length relationship, the general shape of the force-velocity relationship can be observed at all levels from single fiber preparations to voluntary activated whole muscle-joint system (Westing et al. 1990, Finni et al. 2003b, Bobbert 2012). When muscle velocity is negative, i.e. muscle is lengthening, force generation capacity sharply increases and then plateaus with peak values around two times greater than maximal isometric force (Edman 1988). However, in voluntary lengthening contractions, such high eccentric forces may not be reached due to insufficient neural drive (Duchateau & Baudry 2014).

An important implication of muscle's velocity dependence of force generation capacity is that muscle power generation capacity, i.e. the rate at which work can be done, depends on muscle velocity. Power can be calculated as the product of force and velocity. As a result, muscle power generation capacity in shortening contractions peaks at around 0.3 times the maximal shortening velocity (Fig. 7). For maximal eccentric contractions, the power generation increases with increasing speed of lengthening (Roberts 2016).

Unlike the force-length relationship, the force-velocity relationship depends on muscle fiber type (Larsson & Moss 1993, He et al. 2000). The reason for this is that the force-velocity relationship is dependent on the cycling rate of myosin cross-bridges, which is mainly determined by myosin ATPase activity. ATPase activity is higher in fibers expressing fast myosin isoforms capable of faster cross-bridge cycling rate. Consequently, type II muscle fibers can generate greater force at a given shortening velocity (Bárány 1967, Larsson & Moss 1993). This also gives rise to the observation that maximal isometric tension per CSA (specific force), which is not dependent on cross-bridge cycling rate, of different muscle fiber types does not considerably differ, but there is a large difference in power generation capacity (Meijer et al. 2015).

2.2.1.3 Influence of muscle architecture on muscle mechanics

Mechanical function of sarcomeres is constrained by the well-defined force-length and force-velocity relationships. Still, muscles need to be able to cover a

large array of different functional roles. Some muscles need to undergo large excursions and others need to generate high forces. In addition, muscles with different functional requirements need to be packed within constraints set by the skeleton, and preferably be able to cope with functional demands with a minimum amount of muscle mass. Functional versatility of muscles is possible due to different arrangements of the basic contractile building blocks, sarcomeres, i.e. due to different muscle architectures.

The functional importance of muscle architecture can be highlighted by comparison of two hypothetical muscles with the same muscle mass and similar muscle fiber composition. Assuming similar density for the both muscles they also have similar volumes. Muscle force generation capacity is directly related to the number of sarcomeres in parallel and is approximated by PCSA (Wilson & Lichtwark 2011), hence the muscle with shorter fibers has more sarcomeres in parallel and thus greater force generation capacity. On the other hand, the muscle with longer fibers has faster maximal shortening velocity and greater excursion range. Peak power generation for shortening contractions is comparable in both muscles but peak power is achieved with faster absolute shortening velocity in the muscle with longer fibers. The reason for this is that power is calculated as force multiplied by velocity, and force is related to PCSA while velocity is related to fiber length. Thus, power is related to muscle volume since volume can be calculated as PCSA multiplied by fiber length. It should be noted that this hypothetical comparisons does not take in to account the effects of pennation angle and muscle architecture dynamics during muscle length changes (see below for more detailed discussion about these effects) and is thus most appropriate for comparing fusiform muscles with different dimensions. However, this hypothetical comparison shows that the arrangement of muscle contractile tissue in series and in parallel, which is defined by muscle architecture, defines muscle mechanical function to a great extent.

The number of sarcomeres in series defines fiber/fascicle length. Hence fiber/fascicle length has a direct effect on the maximal shortening velocity (Wickiewicz et al. 1984) and shortening range (Winters et al. 2011) that a muscle can obtain. Due to the force-length and force-velocity relationships, fiber/fascicle length also affects force and power generation capacity at a given muscle fiber/fascicle length and velocity. These effects are schematically presented in figure 7. The effect of number of sarcomeres in series on maximal shortening velocity has also been experimentally verified using cat semitendinosus muscle that consists of two anatomically distinct sets of muscle fibers connected in series but having separate innervations. By stimulating these two parts either separately or simultaneously, Bodine et al. demonstrated that whole muscle shortening velocity equaled the sum of shortening velocities of its serially connected parts. Hence, the calculated intrinsic shortening velocity of each sarcomere was constant (Bodine et al. 1982).

A simple geometrical analysis would suggest that pennation angle has a negative effect on whole muscle force generation capacity, since a fraction of muscle fiber force generation is directed perpendicular to the muscle line of action. However, this effect is counterbalanced by the effect of pennation angle on PCSA. Pennation allows greater a number of fibers to be arranged within a

given volume. Hence, a muscle with greater pennation angle at a given volume has greater PCSA (Reeves et al. 2006).

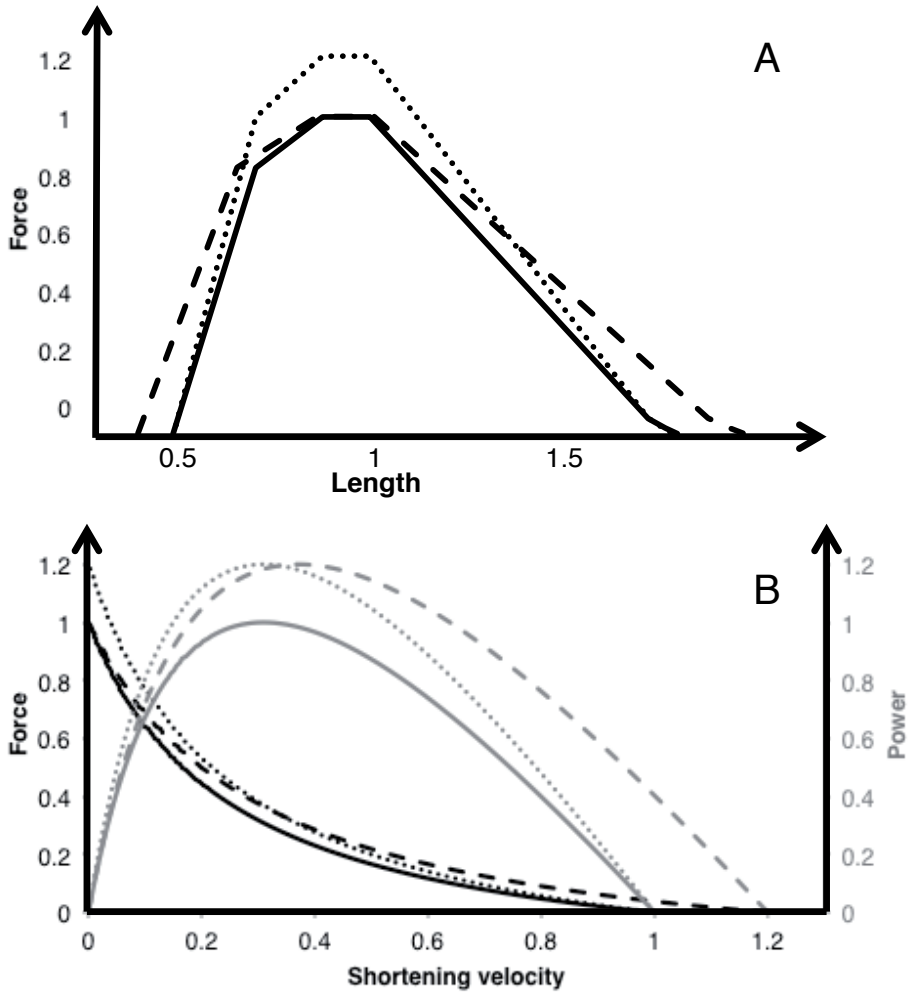

FIGURE 7 Schematic representation of the effects of addition of sarcomeres in series (fascicle length increase) and in parallel (physiological cross-sectional area increase) on muscle's force-length (A) and force-velocity (B) relationships. Dotted lines represent a muscle with 20% greater physiological cross-sectional area and dashed lines a muscle with 20% longer fascicles compared to the reference muscle (solid line). Power-velocity relationships are represented with gray lines in panel B. Scales are normalized to values of the reference muscle.

If one assumes that fascicle pennation angle is fixed, geometrical analysis would reveal that pennation angle has a negative effect on muscle shortening velocity and excursion range. This is due to greater muscle fascicle shortening needed to produce a given muscle shortening (reduction in the distance between distal and proximal MTJ), which is proportional to the cosine of penna-

tion angle. However, in pennate muscles, fascicles rotate, thereby increasing their pennation angle as muscle shortens (Maganaris et al. 1998). Rotation of muscle fascicles amplifies the effect that fascicle shortening has on muscle shortening (Fig 8). The ratio of muscle belly shortening to muscle fascicle shortening is called muscle belly gearing or muscle's gear ratio (Azizi & Roberts 2014). Due to fascicle rotation, muscle belly gearing is actually greater than one, meaning that muscle shortening exceeds that of the fascicle. Since muscle force generation is dependent on shortening velocity (Hill 1938), muscle belly gearing increases the force generation capacity of a muscle by allowing it to contract with slower velocity. In addition, if muscle operates on the ascending limb of the force-length relationship, muscle belly gearing allows for greater force generation for a given joint rotation due to the force-length relationship (Gordon et al. 1966). Muscle belly gearing equals 1/cosine of pennation angle if constant muscle thickness is assumed (Zajac 1989). Thus, muscle belly gearing has a small effect on muscle shortening at pennation angles less than 20°. However, the assumption of constant muscle thickness during contraction is not valid (Randhawa et al. 2013, Randhawa & Wakeling 2013), and changes in thickness also affect muscle belly gearing (Hodgson et al. 2006). Experimentally, muscle belly gearing has been observed to be around 1.05 to 1.1 in human MG and LG during shortening contractions, slightly depending on the level of force generation (Randhawa et al. 2013). In bullfrog plantaris, muscle belly gearing ranged from around 1.2 to 2 in shortening contractions and from 1.8 to 4.3 in lengthening contractions (Azizi & Roberts 2014). High muscle belly gearing during lengthening contractions may protect the muscle against eccentric damage.

Due to the aforementioned reasons, it can be generalized that short fascicle lengths and large pennation angles favor large PCSA per given muscle volume, whereas long fascicles allow high shortening velocity and large shortening range (Ward et al. 2009).

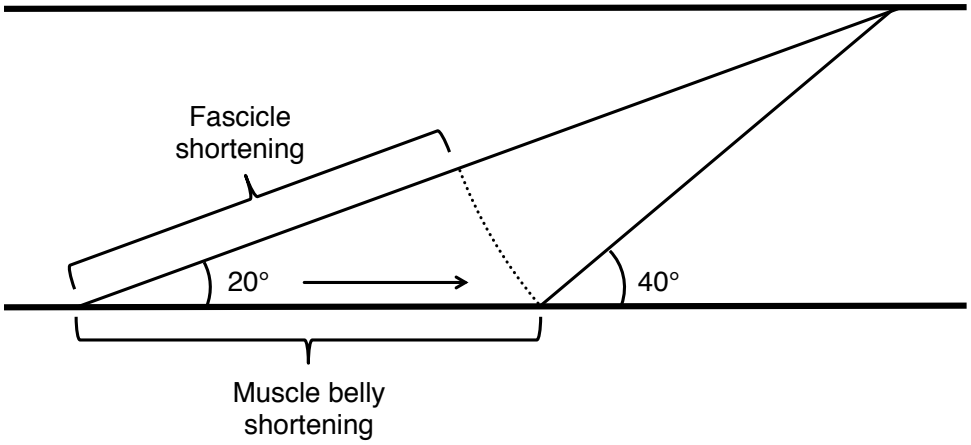

FIGURE 8 An illustration of muscle belly gearing, i.e. the effect that muscle fascicle rotation has on muscle belly and muscle fascicle shortening. Thick horizontal lines represent aponeuroses and thin lines represent a single muscle fascicle at the beginning and end of contraction. At the beginning of contraction, pennation angle is 20° and increases to 40° at the end of contraction. Due to fascicle rotation, muscle belly shortening exceeds the amount of fascicle rotation by 14% if constant muscle thickness is assumed. It should be noted that fascicle rotation can occur due to movement of both the superficial and deep aponeuroses. Here movement is drawn to occur only in the deep aponeurosis for simplicity.

2.2.2 Tendon mechanics

Mechanical properties of a material are measures that describe how the material reacts to physical forces. For tendons these properties are usually measured with so-called tensile tests in which applied deformation and subsequent resisting force are measured. Several parameters can be determined from tensile tests. These include stress and strain at a specific force, stiffness, Young's modulus and hysteresis. Traditionally, tendon tensile tests have been performed on dissected tendons from animals or human cadavers (Maganaris et al. 2008). Improvements in imaging methods have made it possible to perform tendon tensile tests in a physiological environment without invasive procedures by using ultrasonography (Fukashiro et al. 1995) or magnetic resonance imaging (Shin et al. 2008).

Tendons, like many other biological structures, are viscoelastic, meaning that they have both elastic, spring-like properties and viscous, fluid-like properties (Butler et al. 1978). Stiffness and Young's modulus are parameters that describe elastic properties. Hysteresis on the other hand is one manifestation of viscosity in tendon.

Typical data from a tensile test of a tendon shows a curvilinear force-elongation plot (Fig. 9). The initial part of the curve has a lower slope and is called the toe-region. This phenomenon is associated with straightening of the crimped pattern observed in unloaded collagen fibers (Miller et al. 2012). The slope of the force-elongation curve increases as elongation increases. After

straightening of the collagen fibers, a straight line approximates the tendon force-elongation response, hence this region is called the linear region. In this region, tendon elongation may be due to reversible sliding between collagen molecules without structural damage (Svensson et al. 2012, Depalle et al. 2014).

2.2.2.1 Stiffness

When a material, e.g. tendon, is imposed to tensile force it will elongate. The ratio between applied force and elongation ($\Delta F/\Delta L$) is called stiffness (Fig. 9). In order to describe tendon elastic properties stiffness is measured from the linear part of the force-elongation curve.

Tendon stiffness is dependent on length, material properties and CSA of the tendon. For example, if tendon's resting length is doubled, tendon stiffness is halved, since with the same elongation of the tendon, it will resist with half of the force compared to the original situation. On the other hand, if tendon CSA is doubled, tendon stiffness will also double (Butler et al. 1978).

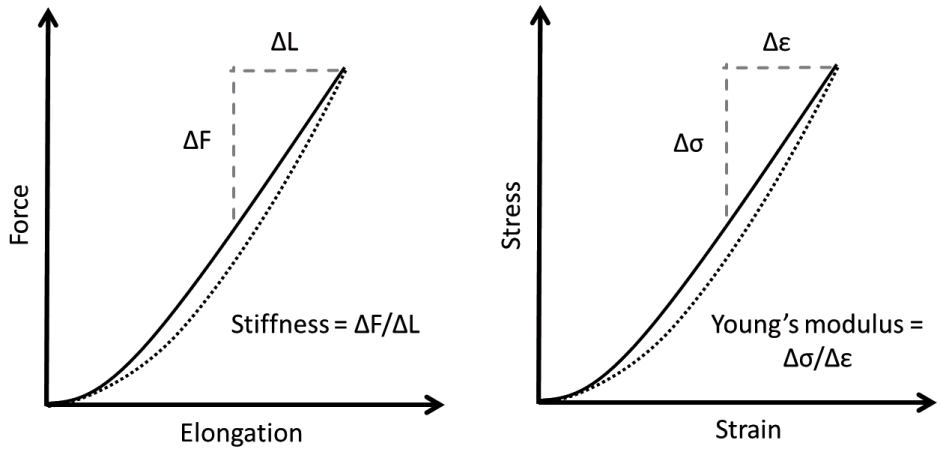

FIGURE 9 Schematic example of typical curves obtained from tensile test of a tendon to determine stiffness, Young's modulus and hysteresis. Solid lines represent loading and dotted lines the unloading phase of the tensile test results. Stiffness is defined as the slope of the force-elongation curve, Young's modulus as the slope of the stress-strain curve, and hysteresis as the percentage of energy lost during a loading-unloading cycle. Note that stiffness and Young's modulus are determined from the linear part of the curves.

2.2.2.2 Young's modulus

If information about mechanical properties of tendon as a material is to be measured, tensile test results needs to be normalized to the dimensions of the tendon. A variable that normalizes tendon stiffness to its dimensions is called Young's modulus. Where stiffness is a measure of a material's force-elongation relationship, Young's modulus is a measure of a material's stress-strain rela-

tionship. Stress is calculated by dividing tensile force by CSA ($\sigma = F/A$), and strain is calculated by dividing elongation by initial length ($\varepsilon = \Delta L/L_0$). Both stress and strain are independent of dimensions of the material tested, hence Young's modulus does not depend on the dimensions but only on the properties of the material (Butler et al. 1978). This makes Young's modulus a useful parameter for comparisons between individuals or different tendons.

2.2.2.3 Hysteresis

Although tendons have been described to behave almost perfectly elastically (Elliott 1965), they still show some degree of viscous characteristics when physically loaded, giving tendon it's viscoelastic properties. Hysteresis is one of the manifestations of tendon viscosity.

A loop is formed in the force-elongation or stress-strain curve when tendon is cyclically loaded. This loop is called hysteresis, and reflects the amount of energy converted to heat during the loading cycle. Hysteresis is typically expressed as a percentage, and calculated as the area (energy) between the loading and unloading curves relative to the area under the loading curve (Maganaris et al. 2008). Hysteresis values ranging from 3 to 38 % have been reported in vivo (Finni et al. 2012). The amount of hysteresis may be lower in high stressed tendons that have a role in energy storage and release compared to low stressed tendons of positional muscles (Shadwick 1990). For example, elastic properties have been shown to prevail over viscous properties in Achilles tendon in vivo, giving rise to a relatively low hysteresis (Peltonen et al. 2013).

2.2.3 Muscle-tendon interaction

MTU mechanical function is determined by interaction between muscle and passive series elastic components. Passive elastic components include intramuscular components like cross-bridges, actin, myosin and titin filaments, and collagen fibers in the extracellular matrix. However, tendon and aponeurosis have the greatest capacity for elastic energy storage (Roberts 2016), and thus can be considered the most important elastic component regarding MTU function. Quick release experiments combined with high frame rate ultrasound imaging support this as they have shown that outer tendon makes the largest contribution to MTU series elasticity, but also aponeurosis has a significant role (Farcy et al. 2014).

In biomechanical studies of MTU function, a mechanical model in which tendon and aponeurosis (collectively called tendinous tissue, TT) are connected in series with muscle fascicles has been widely used. In this model muscle fascicles are lumped to a single contractile component representing all muscle fascicles in parallel (Fukunaga et al. 2001). This model is useful for in vivo studies since it allows estimation of length changes in passive series elastic components (named TT in this model) based on joint kinematics and measurement of a single muscle fascicle length. However, it should be noted that TT is not specific tissue or combination of tissues but rather a model representation of series elastic components. Most of the studies reviewed in this chapter have used the

muscle model like described above. Hence, for clarity TT is used throughout the chapter when referring to passive series elastic components.

As described previously in chapter 2.2.1, mechanical function at the muscle fascicle or fiber level is constrained by the force-velocity (Hill 1938) and force-length relationships (Gordon et al. 1966), with the arrangement of muscle fibers (muscle architecture) expanding functional versatility of muscles. Mechanical function of TT on the other hand is determined by its mechanical properties such as stiffness. The way active muscle and passive TT interacts to produce MTU mechanical function is the key to functionally distinct MTUs that can fulfill a large array of different functional demands (Wilson & Lichtwark 2011). For example, elasticity of TT can be utilized to decouple muscle length and velocity from that of MTU (Fukunaga et al. 2001), and thereby to overcome limitations set by a muscle's force-velocity and force-length relationships. Regarding energetics of MTU, TT may have three different roles: energy conservation, power amplification and power attenuation (Roberts & Azizi 2011). These and other effects of muscle-tendon interaction on MTU function are reviewed in this chapter.

2.2.3.1 Energy conservation

Energy conservation by utilizing TT elasticity is an important mechanism to improve efficiency of human locomotion. The basic mechanism of energy conservation by TT is that kinetic and potential energy related to movement can be temporarily stored in TT and subsequently released to power the movement (Cavagna et al. 1977). A typical example of energy conservation using TT elasticity is a bounding gait such as running at constant speed. In running, kinetic and potential energy reach minimum values during the stance phase and maximal values during the flight phase (Novacheck 1998). Based on measurements of energy consumption and external mechanical work in a range of animals and in humans, Cavagna et al. concluded that at least some of the kinetic and potential energy lost during ground contact must have been stored temporarily in elastic structures and subsequently returned, thereby improving efficiency of locomotion (Cavagna et al. 1964, Cavagna et al. 1977). Using direct measurements of tendon force and fascicle length in hopping wallabies, Biewener et al. showed that two distal hindlimb muscles, plantaris and gastrocnemius, do minimal positive work during a hopping cycle. In contrast, energy recovered from TT was on average 20 times greater than muscle work output (Biewener et al. 1998). This basic mechanism of energy cycling using TT elasticity has been confirmed later in humans with ultrasound based measurements of muscle fascicle and TT behavior in running (Lichtwark et al. 2007, Lichtwark & Wilson 2006, Lai et al. 2015, Farris & Sawicki 2012) and hopping (Hoffrén et al. 2012). In summary, the cornerstone of improving efficiency of locomotion by utilizing TT elasticity is the energy cycling between the body and TT elastic energy. This allows muscle to do either no external work or to work at a slow shortening velocity, when efficiency is greatest (He et al. 2000).

Although muscle may not do mechanical work, this does not mean that energy cost would be zero. Storing elastic energy in TT requires the muscle to

generate force. However, as energy cost of force generation is lower in isometric compared to concentric contractions (Fletcher et al. 2013a), energy conservation utilizing TT elasticity may create energy saving compared to a situation where MTU work would have to be performed by the muscle. It should be noted however that one study performed on frog muscles contradicts the common assumption that replacing muscle work with tendon work improves efficiency of locomotion. In this study, Holt et al. found that cost of force generation was similar in a condition were TT was responsible for MTU length changes and in a condition where muscle itself performed stretch-shorten cycles (Holt et al. 2014). Thus, alternative explanations of energy savings utilizing elastic TT may be formulated. Firstly, it may be that a long tendon and proximally situated muscle reduce limb inertia, thereby reducing energy cost associated with limb movements. Secondly, short and highly pennate muscle fascicles that are associated with long tendons may reduce energy cost by minimizing the amount of muscle mass activated per unit of force generation (Roberts 2016).

2.2.3.2 Power amplification

Muscles generate peak positive power at a shortening velocity around 20-30% of the maximal shortening velocity (Askew & Marsh 1998, He et al. 2000). In some movements, fast joint rotations occur where MTU shortening velocity greatly exceeds optimal velocities for muscle power generation. In such fast movements, joint power can exceed what would be theoretically possible for the muscle even if contracting with optimal velocity (Sawicki et al. 2015). This high power generation exceeding muscle capacity is called power amplification.

Power amplification may occur when energy stored in TT is suddenly released to power limb movement. Some animals utilize an anatomical catch mechanism to allow muscle to stretch the tendon with a slow shortening velocity before the start of the movement. This is similar to the operation of a catapult. Humans can use body mass and limb inertia to achieve the same without an anatomical catch (Farris et al. 2015). Such a mechanism is in play in countermovement jumps and in walking. Kurokawa et al. (2001) showed that prior to takeoff in a squat jump, MG muscle fascicles shortened while MTU length stayed constant, storing elastic energy in TT. During MTU shortening, MG muscle fascicles generated force almost isometrically and TT was responsible for almost all MTU power generation. Here, high force generation by triceps surae muscles prior to ankle joint movement initiation is possible by acceleration of body segments proximal to the ankle joint, allowing increase in ground reaction force generation and energy storage in Achilles tendon. In human walking, a similar mechanism can be utilized. Gastrocnemius and soleus muscles can generate force and elongate TT while the shank rotates forward during stance. During push-off, TT shortens increasing MTU power output above muscle power output and allowing high positive ankle joint power to be generated during late push-off (Ishikawa et al. 2005).

2.2.3.3 Power attenuation

Since TTs are passive and generally have low hysteresis (Ker 1981), they cannot dissipate large amounts of energy in MTUs. Thus, muscle is the component that can absorb energy in a MTU. TTs can however attenuate power, i.e. decrease the rate at which muscle needs to absorb energy. Power attenuation is needed, e.g. in landing situations where the body's mechanical energy rapidly decreases. Muscle dissipates energy by eccentric contractions, i.e. doing negative work. Here, active muscle contraction converts mechanical work to heat. Without elastic TT, dissipation of a large amount of energy in a short time would require a forceful eccentric contraction that could expose the muscle to a risk of eccentric muscle injury (Thelen et al. 2005). Energy can be transiently stored in TT and then dissipated by the muscle with a slow eccentric contraction, where muscle force does not need to rise to extreme values. Thus, the benefit of the slow eccentric velocity in energy dissipation situations is that it may protect musculoskeletal structures from damage by reducing peak forces and muscle lengthening velocities (Roberts & Azizi 2010).

2.2.3.4 Modulation of muscle-tendon interaction

In dynamic contractions muscle activation timing critically determines MTU power output and TT function. Changes in muscle activation timing and intensity during cyclic MTU length changes can alter the function of TT and change MTU function from power amplification to power attenuation (Sawicki et al. 2015a, Lichtwark et al. 2009). In addition, TT stiffness plays a significant role in muscle-tendon interaction. Both simulation and experimental studies have shown that MTU power generation and efficiency depend on TT stiffness (Lichtwark & Wilson 2005b, Lichtwark & Barclay 2010), which is probably caused by the effect that TT elasticity has on muscle fiber shortening velocity. In addition, in simulated walking, muscle fiber velocity and length are sensitive to TT stiffness (Arnold & Delp 2011). Different tasks, such as walking and running with different speeds, may optimally require different stiffness in triceps surae TT (Lichtwark & Wilson 2008). Aponeurosis may play a role in tuning TT stiffness appropriately for different tasks. Longitudinal stiffness of an aponeurosis has been shown to vary according to muscle force generation (Azizi & Roberts 2009). This may allow for tuning TT stiffness and thereby improving MTU functional versatility. Finally, in vivo human studies have shown associations between TT stiffness and running performance (Kubo et al. 2000, Kubo et al. 2015) and economy (Fletcher et al. 2013b), and changes in triceps surae TT stiffness are associated with changes in running economy (Fletcher et al. 2010, Albracht & Arampatzis 2013).

2.2.3.5 Rate of force development

Series elasticity also has an effect on MTU rate of force development. In fixed end contractions (i.e. isometric), the greater the elongation of series elastic components with maximal tension of the muscle, the slower will be the maximal rate of force development of the MTU. Wilkie demonstrated this effect in 1949

by adding compliance in a cable extending from the hand to a dynamometer for an elbow flexion task (Wilkie 1949). The same effect of compliance has also been observed for knee extensors. Bojsen-Møller et al. (2005) showed that patella tendon stiffness is positively associated with knee extension rate of torque development. This effect is thought to derive from muscle's force-velocity relationship since in fixed-end contractions, there is shortening in the muscle due to stretch of TT until peak force generation is reached (Roberts & Azizi 2011). The smaller is the shortening distance, the faster the peak force can be reached. Hence, the effect of series elasticity on rate of force development is greatest in MTUs where tendon to muscle length ratio is large.

2.2.3.6 Force-length relationship

One of the effects of TT elasticity is that it distorts the shape of muscle force-length relationship compared to that of sarcomere force-length relationship if measured with fixed-end contraction of the MTU. In fixed-end contractions, TT elongates by an amount that is determined by TT stiffness and muscle force generation and since MTU length remains constant, muscle will shorten by the same amount than TT elongates. The net effect of the muscle shortening is a rightward shift and distortion in the shape of the force-length relationship that is dependent on series elastic component properties (Lieber et al. 1992). The effects of TT elasticity on the force-length relationship have also been observed in vivo as a shift in the isometric and isokinetic knee extension torque-angle relationships with different levels of torque generation due to different amount of TT lengthening in these different conditions (Kawakami et al. 2002, Kubo et al. 2006).

2.3 Muscle architecture, tendon properties and muscle-tendon interaction in aging

No longitudinal studies have examined changes occurring with aging in muscle architecture or tendon properties. Thus, current knowledge regarding possible age-related changes relies on cross-sectional observations. Muscle architecture, as well as tendon mechanical and morphological properties, respond to different levels of physical loading (Blazevich 2006, Magnusson et al. 2008, Bohm et al. 2015, Wiesinger et al. 2015, Timmins et al. 2016, Ema et al. 2016). Thus, results from cross-sectional studies comparing individuals of different ages have to be viewed in light of the inherent limitations of the cross-sectional study design, i.e. its vulnerability to bias from confounding factors. In particular, the confounding effect of differing levels of physical loading of tissues in different age groups needs to be acknowledged, and causality cannot be interpreted based on cross-sectional observations. However, the effects of aging take considerable time to be observable, and cross-sectional designs provide a practical method to probe possible changes occurring with aging.

2.3.1 Aging and muscle architecture

It is well documented that aging is associated with a decline in muscle mass. There is a lack of long-term longitudinal studies, but estimates based on cross-sectional observations indicate a decline in muscle mass of around 4-5% per decade, representing a decline of about 15% to 20% from the age 30-40 (the approximate age of peak muscle mass) by the age of 70-80 (Mitchell et al. 2012). However, it should be noted that high variation exists between studies in the rate of decline in muscle mass with aging based on cross-sectional observations. Values ranging from 8-49% in individuals aged from 18 to 80 have been reported (Mitchell et al. 2012). The loss of muscle mass with aging can be a result of reductions in contractile tissue mass in series and in parallel, and thus alterations in muscle architecture are expected with aging.

Table 1 summarizes cross-sectional observations of age-related differences in muscle architecture from in vivo human studies. Typically in these studies young adults were approximately 20-30 years old and older adults over 70 years old. However, in some studies people aged 60-70 were included in older group. From the table it can be seen that on average the studies have reported 8% lower muscle fascicle lengths, 14% lower pennation angles and 16% lower muscle thicknesses in older adults compared to young adults across muscles. Statistically significant differences between young and older adults were reported in 1/3, 1/2 and 2/3 of the studies for fascicle length, pennation angle and muscle thickness, respectively.

The presented studies are generally consistent in the direction of the age-related differences in fascicle length, pennation angle and muscle thickness. As expected, none of the studies have reported significantly greater values in these parameters in older compared to young adults. However, the magnitude of the difference between groups varies across studies, which may be due to differences in age or physical activity levels of the subjects. As the results consistently show declines in the abovementioned muscle architectural parameters, the lack of significant difference in most studies is probably due to small sample sizes and large individual variation, leading to insufficient statistical power to observed differences between the groups.

TABLE 1 Percent differences in muscle architecture in older compared to young adults from in vivo human studies.

Fascicle length	Pennation angle	Muscle thickness	Muscle	Reference
-17	-32		MG	(Sinha et al. 2014)
-10*	-13*		MG	(Narici et al. 2003)
-16*	-13*		MG	(Morse et al. 2005b)
-19*			MG	(Thom et al. 2007)
	-5	-14*	MG	(Kubo et al. 2003)
		-13*	MG	(Onambele et al. 2006)
3	-3	-9	MG	(Karamanidis & Arampatzis 2006)
0	-14*	-9*	MG	(Randhawa & Wakeling 2013)
8	-24*	-18*	LG	(Randhawa & Wakeling 2013)
-9	-18*		LG	(Morse et al. 2005b)
-10	-14		LG	(Sinha et al. 2014)
-16	-15*		Soleus	(Morse et al. 2005b)
	-5	-6	TB	(Kubo et al. 2003)
		-16*	Plant.flex.	(Kubo et al. 2007a)
		-7*	Plant.flex.	(Kubo et al. 2007b)
-5	-2	-12	VL	(Karamanidis & Arampatzis 2006)
-9*	-27*		VL	(Power et al. 2013a)
0	-11*		VL	(Suetta et al. 2009)
	-20*	-23*	VL	(Kubo et al. 2003)
		-45*	Knee ext.	(Kubo et al. 2007a)
-8	**-14**	**-16**	**Average**	

Asterisks denote statistically significant difference between young and older adults. The results are from pre-intervention measurements or from mid muscle if applicable. Plantar-flexor and knee extensor refers to a summary value of the muscle group.

Fascicle length reflects the amount of contractile tissue in series, and thus changes in fascicle length are due to changes in the amount of contractile tissue (i.e. sarcomeres) along the fibers. Changes in pennation angle on the other hand are thought to reflect changes in muscle fiber CSA (Gans & Bock 1965) and thus the number of sarcomeres in parallel. The reduction in pennation angle with loss of sarcomeres in parallel may provide an intrinsic compensatory mechanism, as a greater proportion of fascicle force is transmitted to tendon with lower pennation angles (Blazevich 2006). If muscle fascicle length is unaltered, a change in muscle thickness is expected with a change in pennation angle. Based on the observations of age-related differences in muscle architecture presented in table 1, it seems that in aging, the reduction in contractile tissue in parallel is greater compared to the reduction in series.

2.3.2 Aging and tendon properties

Aging has been reported to be associated with differences in several aspects of tendon tissue, from cellular and molecular to whole tendon level differences. Here the literature is reviewed in relation to tendon CSA and whole tendon mechanical properties.

Cross-sectional observations in humans have not provided conclusive results about age-related differences in tendon mechanical and morphological properties. Results from in vivo human studies regarding age-related differences in tendon stiffness, Young's modulus and CSA are presented in table 2. About half of the studies reported here have found statistically significant differences in tendon stiffness or Young's modulus between young and older adults. However, none of the studies have found greater stiffness or Young's modulus in older compared to young adults, which differs from the findings of some animal studies (Tuite et al. 1997, Nielsen et al. 1998, Wood et al. 2011). If statistical interpretation is neglected, on average studies have found 25% greater tendon stiffness and Young's modulus in young compared to older adults. Based on these observational studies, age-related differences in lower limb tendon mechanical properties are inconclusive but suggest a decline in stiffness and Young's modulus with aging. The similar magnitude of difference between young and older adults in both stiffness and Young's modulus suggests that the decline in stiffness with aging is mainly due to a decline in tendon material properties.

For tendon CSA, cross-sectional human studies show conflicting results. From table 2 it can be seen that four studies have reported significantly larger and one study significantly smaller tendon CSA in older compared to young adults in apparently healthy tendons. Results averaged across studies suggest a modest increase in tendon CSA associated with aging. Tendon-specific (Achilles or patella) differences between the studies do not seem to be the reason for the conflicting results. Methodological differences could play a role in the variation of results between studies since some studies have used ultrasound imaging (Onambele et al. 2006, Pang & Ying 2006, Tweedell et al. 2016) and some used magnetic resonance imaging (Magnusson et al. 2003a, Carroll et al. 2008, Couppé et al. 2009, Couppé et al. 2012, Couppé et al. 2014). Another possible reason for the discrepancy between studies could be differences in measurement points along the tendon, since region-specific CSA adaptations have been observed after resistance training interventions (Bohm et al. 2014, Kongsgaard et al. 2007) and habitual loading (Magnusson & Kjaer 2003). Similar region specificity may also be present in age-related modifications. Finally, differences in the characteristics of subject groups, such as physical activity levels, age or unnoticed pathologies, between studies could also play a role in the discrepant results.

In addition to the tensile test results reported in table 2, age-related differences in tendon mechanical properties have been studied using shear wave elastography. The results of these studies are inconclusive as one study found greater shear wave speed in Achilles tendon in older compared to young adults (Turan et al. 2015), and other reported similar shear wave speed in middle-aged compared to young adults (Slane et al. 2015). However, the latter study found lower shear wave speed in gastrocnemius aponeurosis in middle-aged compared to young adults suggesting lower aponeurosis stiffness in the older group.

TABLE 2 Percent differences in tendon stiffness and Young's modulus in older compared to young adults from in vivo human studies.

Stiffness	Young's modulus	CSA	Tendon	Reference
-39*	-28*	-19*	Achilles	(Onambele et al. 2006)
-55*	-52*	4	Achilles	(Csapo et al. 2014)
		22*	Achilles	(Magnusson et al. 2003a)
-36*			Achilles	(Hoffrén et al. 2012)
		46*	Achilles	(Tweedell et al. 2016)
		22*	Achilles	(Pang & Ying 2006)
-2	-25*	13*	Patella	(Couppe et al. 2014)
-6	0	-8	Patella	(Carroll et al. 2008)
-29	-23	2	Patella	(Couppé et al. 2009)
-15	-23	-5	Patella	(Couppé et al. 2012)
-30*			Plantarflex.	(Mademli & Arampatzis 2008)
-4†			Plantarflex.	(Karamanidis et al. 2008)
-36†			Plantarflex.	(Karamanidis & Arampatzis 2006)
-31†			Knee ext.	(Karamanidis & Arampatzis 2006)
-25*†			Knee ext.	(Karamanidis et al. 2008)
-25	**-25**	**9**	**Average**	

Asterisks denote statistically significant differences between young and older adults. The results are from untrained groups or from pre-intervention measurements if applicable. Statistical significance is evaluated from main effects in case of factorial design. Achilles and patella tendon includes only outer, tendon whereas plantarflexor and knee extensor results include outer tendon and part of the intramuscular aponeurosis. The results marked with daggers were derived from force-strain relationship instead of conventional force-elongation relationship.

2.3.3 Aging and muscle-tendon interaction

The age-related differences observed in muscle architecture and tendon mechanical properties suggest that there may be age-related differences in muscle-tendon interaction. However, studies in this area are scarce.

Two previous studies have examined age-related differences in muscle-tendon function of triceps surae muscle during walking. Mian et al. (2007) found that when young and older adults were compared at matched walking speeds, older adults showed less LG fascicle elongation during MTU lengthening consistent with the previously reported age-related decrease in Achilles tendon stiffness. Panizzolo et al. (2013) showed that soleus fascicle behavior was similar between young and older adults when compared at their preferred walking speeds. Increase in walking speed shifted soleus operating length to a shorter length consistent with greater Achilles tendon elongation, since ankle joint kinematics were unaltered. This finding suggests adaptation of preferred walking speed in older adults to preserve soleus muscle fascicle mechanical behavior.

(Morse et al. 2005a) found greater elongation of TT in plantarflexors during maximal voluntary contractions in older compared to young adults, which was associated with longer electromechanical delay in older adults. The authors

speculated that longer electromechanical delay could affect postural control in older adults. This was supported by the findings of Onambele et al. (2006) showing that Achilles tendon stiffness was associated with measures of postural balance in older adults.

Hopping and drop jumps have been used as a model to study neuromuscular control and muscle-tendon interaction in young and older adults in triceps surae muscles (Hoffrén et al. 2007, Hoffrén et al. 2012). These studies have shown differences in muscle-tendon interaction between the age groups during drop jumps. The differences arise mainly from differences in fascicle behavior, suggesting adaptation of TT stiffness to match the level of muscle force generation capacity. Results from hopping tasks further supported the adaptation hypothesis, since muscle-tendon interaction was shown to be similar between young and older adults in conditions that required similar relative effort, but not in conditions that were matched in absolute terms.

TT stiffness in triceps surae muscles may adapt to loading encountered in daily living where muscle actions are typically isometric or concentric (Farris & Sawicki 2012). Barber et al. (2013) showed that in maximal isokinetic eccentric contractions, older adults had preserved torque generation, although significantly lower values were observed in isometric and concentric contractions compared to young adults. Eccentric contractions were associated with lower MG lengthening velocity in older compared to young adults suggesting that Achilles tendon elongation accounted for a greater proportion of lengthening of the MTU in older adults. No such difference was observed in concentric contractions.

3 PURPOSE OF THE STUDY

Previous cross-sectional observations suggest that aging may have an effect on muscle architecture and tendon mechanical properties. However, the evidence is not conclusive with varying and even contradictory findings. One of the reasons for the inconsistent findings may be varying levels of physical activity between the subject groups studied.

One drawback of cross-sectional studies is that they are affected by confounding factors. Using a cross-sectional study design, it is difficult to separate the effects of primary aging from those of declining physical loading, since aging is typically associated with a decline in physical activity levels (Ayabe et al. 2009) and an increase in sedentary behavior (Wullems et al. 2016). It has been suggested that master athletes provide a model of exceptionally successful biological aging due to continued high levels of physical activity and few comorbidities (Tanaka & Seals 2008).

The age-related loss of muscle mass is a major factor in the age-related decline in muscle function (Frontera et al. 1991). However, longitudinal studies have shown a disproportionately larger loss of muscle strength compared to loss of muscle mass (Delmonico et al. 2009, Goodpaster et al. 2006). Muscle architecture and tendon mechanical properties have profound effects on muscle function and could partially explain the disproportionately greater loss in muscle function compared to mass. Comparative studies contrasting different muscles or different species with distinct muscle functional roles have enhanced our knowledge of the functional significance of muscle-tendon properties (Alexander & Bennet-Clark 1977, Lieber & Friden 2000, Gillis & Biewener 2001, Biewener 1998, Biewener et al. 1998). However, the effects of subtle changes in muscle-tendon properties, such that may occur with aging, are relatively unknown.

Walking is the most common form of mobility, and age-related impairments in walking speed are highly predictive of future health-related outcomes (Albert et al. 2014, Montero-Odasso et al. 2005). The most profound age-related impairment in walking is reduced ankle plantarflexion power in late push-off (Kerrigan et al. 1998, DeVita & Hortobagyi 2000, Kulmala et al. 2014). Using ultrasound imaging it has been shown that plantarflexors utilize TT elasticity

for high power generation in the push-off phase of walking (Ishikawa et al. 2005). Due to a large tendon to muscle length ratio, muscle function in plantar-flexors is highly dependent on tendon mechanical properties (Zajac 1989). For that reason, age-related changes in Achilles tendon properties could play a role in the previously reported age-related impairments in walking.

Therefore, the purpose of this thesis was to increase knowledge on the association between aging and muscle architecture, tendon mechanical properties and muscle-tendon function of human triceps surae muscle.

Specifically the aims of the current thesis were:

1) To describe age-related differences in triceps surae muscle architecture and selected Achilles tendon properties in healthy young and older adults (I).

2) To examine the association between physical function and triceps surae muscle-tendon properties in healthy older adults to reveal the functional relevance of inter-individual differences in muscle architecture and tendon properties (II).

3) To gain insight into the association between aging and triceps surae muscle-tendon properties in healthy aging without the confounding by lack of physical loading (III). This was achieved by studying older athletes. Two groups of athletes were studies, older endurance and sprint runners.

4) To examine age-related differences in triceps surae muscle-tendon function during walking in order to understand the mechanistic role of age-related differences in muscle architecture and tendon properties in age-related impairments of physical function (IV).

4 METHODS

4.1 Study design and subjects

4.1.1 Study design

A cross-sectional study design was utilized with healthy young (18-31 years old) and older (70-80 years old) adults studied.

In paper I, triceps surae muscle architecture and Achilles tendon mechanical and morphological properties were measured from young and older men and women and compared between the age groups.

In paper II, association between triceps surae muscle-tendon properties and physical function was examined in older adults using regression modeling.

Paper III compared triceps surae muscle architecture and Achilles tendon mechanical and morphological properties between older athletes and untrained young and older adults.

Paper IV compared triceps surae muscle-tendon function between young and older adults during walking. In this study, subjects walked over a 10-meter force platform at their preferred walking speed while muscle-tendon function of MG and soleus was examined using ultrasound imaging. In addition to the preferred walking speed, older subjects performed trials at a speed that matched the mean preferred speed of young subjects.

The author of the current thesis was responsible for all the measurements excluding physical function and body composition measurements of original paper II. The author analyzed all data and was the first author of the original papers, reflecting a major role in drafting and editing the manuscripts.

4.1.2 Subjects

A total of 146 healthy young and older people participated in the studies that made up the four original papers included in this thesis. Papers I and II include untrained healthy young and older adults that were participating in a Europe-wide cross-sectional study called MyoAge. Young and older men from this da-

taset were also included in paper III alongside 20 older athletes. Paper IV included 13 young and 13 older untrained healthy men. Age and physical characteristics of the subjects are presented in table 3.

TABLE 3 Subject characteristics and the original paper in which the subjects appear.

	Age (yr)	Height (m)	Body mass (kg)	Original paper
Young				
Women (n=15)	25 ± 3	166 ± 4	63 ± 6	I
Men (n=18)	24 ± 2	181 ± 6	75 ± 9	I, III
Older				
Women (n=34)	74 ± 3	159 ± 5	64 ± 9	I, II
Men (n=33)	75 ± 4	173 ± 5	76 ± 8	I, II, III
Older athletes				
Endurance runners (n=10)	74 ± 3	175 ± 7	70 ± 7	III
Sprint runners (n=10)	74 ± 3	176 ± 7	74 ± 7	III
Young men (n=13)	25 ± 4	179 ± 6	79 ± 7	IV
Older men (n=13)	73 ± 5	176 ± 5	88 ± 9	IV

Values are presented as mean ± standard deviation.

The aim of subject recruitment was to obtain a sample of healthy young and older adults free from major diseases. Young adults were recruited among students of the University of Jyväskylä using email bulletins. Older adults, excluding the older athletes, were recruited from local meetings of retired people and from lectures organized by University of the Third Age.

Older athletes in paper III were recruited among the participants of the World Master Athletics Indoor Championships held in Jyväskylä, Finland in 2012. Twenty male athletes were recruited based on the events they participated in during the championships. Ten subjects were recruited from sprint running events (60 m, 60 m hurdles, 200 m, 400 m) and 10 were recruited from endurance running events (3 km, ½ marathon and 8 km cross country running). The rationale behind the recruitment was that these groups load their triceps surae muscles with distinct loading patterns: one group with high volume and lower intensity, and the other with low volume but high intensity.

All subjects were community living, moderately socially active (participating in social or group activities to improve knowledge or skills two times or more per month), free from major diseases and did not have mobility limitations that would prevent them from walking 250 m without assistance. For details of the recruitment and exclusion criteria used, readers are referred to the original papers.

4.1.3 Ethics

Participants were informed about the procedures used in the study and they all signed a written consent prior to participation in the study. The ethics committee of the Central Finland Health Care District (I-III) or the University of

Jyväskylä (IV) approved all methods and the studies conformed to the standards set by the latest revision of the Declaration of Helsinki.

4.2 Data collection and analyses

All analog data (force, electromyography, potentiometer and goniometer data) were sampled with a 16-bit A/D board (Power 1401, CED Limited, England) with a sampling rate of at least 1000 Hz. Data analyses were performed with custom scripts written in MATLAB (MathWorks Inc., Natick, MA, US). All ultrasound data were saved to a computer for offline analyses.

4.2.1 Achilles tendon cross-sectional area (I-III)

Achilles tendon CSA was measured from the right leg in a relaxed condition while subjects lay prone on a table with ankle angle at 90°. A linear 3.6 cm ultrasound probe (Aloka Pro Sound alpha 10 and UST-5411, Aloka, Japan) and an acoustic gel pad (SonarAid, Geistlich Pharma, Switzerland) were used. Ultrasound image of Achilles tendon CSA were taken in the transverse plane, four centimeters proximal to the proximal margin of the calcaneal tubercle (Achilles tendon insertion site), which is approximately the narrowest site of the free Achilles tendon (Peltonen et al. 2010).

Tendon CSA was measured by manually outlining the tendon (ImageJ, National Institute of Health, USA). The measurement was performed twice and the average was used in subsequent analyses.

4.2.2 Triceps surae muscle architecture (I-III)

To measure muscle architectural parameters, single ultrasound images were taken from MG and soleus at the level of 50% of MG length and mid muscle in the medial-lateral direction using a 6 cm linear probe (Aloka Pro Sound alpha 10 and UST-5712, Aloka, Japan). The images were taken with the subjects in the same position as in the Achilles tendon CSA measurements. The probe was carefully oriented along the direction of fascicles with minimal pressure applied to the skin.

Anatomical CSA was measured from gastrocnemii by combining MG and LG. The CSA was measured at 50% of MG length using panoramic ultrasound imaging with a 3.6 cm linear probe (Aloka Pro Sound alpha 10 and UST-5411, Aloka, Japan). The panoramic image was formed by a combination of several images taken as the ultrasound probe was moved over the muscles. The ultrasound device combined these images and the panoramic image was saved to a computer for later analysis. Soleus muscle was not included in the muscle CSA measurement because the borders of the muscle were not clear in the panoramic images. Panoramic ultrasound imaging has been found to be valid and reliable for measurement of muscle anatomical CSA (Ahtiainen et al. 2010, Noorkoiv et al. 2010).

Muscle thickness was measured as the distance between the deep and superficial aponeuroses perpendicular to the deep aponeurosis in the middle of the image. Muscle fascicle length was measured by drawing a line along a clearly visible muscle fascicle between the deep and superficial aponeuroses. If the fascicle was not apparently straight, the curvature of the fascicle was taken into account by drawing the line with multiple points. Pennation angle was measured as the angle between muscle fascicle and deep aponeurosis. Anatomical CSA was measured by manually outlining the muscles. All measurements were performed twice and the average was used in subsequent analyses (ImageJ, National Institute of Health, USA).

4.2.3 Plantarflexion strength (I-IV)

Plantarflexion strength was measured in a custom-built ankle dynamometer (University of Jyväskylä, Finland) with maximal voluntary isometric contractions (MVIC) of the right leg. Subjects were seated in the dynamometer with the right leg ankle at 90° of flexion, knee fully extended and hip at 60° of flexion (full extension 0°). The seat was set individually as close to the pedal as possible to minimize ankle joint rotation during maximal plantarflexion efforts. Force applied by the subjects to the dynamometer footplate was measured with a force transducer (Precision TB5-C1, Raute, Nastola, Finland) installed to the pedal of the dynamometer. After a standardized warm-up, three maximal trials were performed with one-minute rest between the trials. If maximal force was greatest in the third trial, subsequent trials were performed until maximal force did not increase. The highest measured force was taken as plantarflexion strength. Force instead of ankle joint torque was used as a measure of plantarflexion strength since it was considered to reflect the maximal functional force (e.g. ground reaction force at the level of the metatarsal heads) that the plantarflexor muscles could generate. Plantarflexion muscle strength was normalized by dividing it by body weight.

4.2.4 Achilles tendon mechanical properties (I-III)

Achilles tendon mechanical properties were measured in the same dynamometer with the same joint ankle positions that were used for plantarflexion strength measurements. A combination of synchronous ultrasound imaging, video based motion capture, force measurements and heel displacement measurements were used to derive Achilles tendon force-elongation and stress-strain curves (Peltonen et al. 2013).

After practicing the correct rhythm for the contractions, subjects performed a set of five to eight fast isometric ramp contractions with ankle plantarflexors. Each contraction in the series lasted for one second with the rise and fall of force occurring at approximately the same rate. The peak force reached in each contraction was at least 80% of MVIC force. This kind of contraction has been shown to provide better reproducibility of force generation than slower contractions without affecting tendon stiffness (Peltonen et al. 2013). Practice trials and MVIC measurements performed before the measurement of Achilles

tendon mechanical properties were considered to precondition the tendon and stabilize its mechanical properties (Maganaris 2003b).

Achilles tendon force was calculated by multiplying the measured reaction force by the ratio between the externally measured lever arms of the foot and the Achilles tendon. To measure the foot lever arm, subjects placed their right foot on to a piece of paper that had a scale printed on it. The longitudinal foot axis was perpendicular to the scale. The vertical projections of the outermost tip of medial malleolus and the head of the first metatarsal were marked on to the paper and the distance between these points was determined as the lever arm of the foot. Achilles tendon lever arm was defined as the distance from the center of the Achilles tendon to the outermost tip of the medial malleolus in the sagittal plane measured using a ruler. There was no difference in Achilles tendon loading rate between young and older adults ($p=0.440$).

Tendon length during the trials was measured using a combination of ultrasound imaging of MG MTJ, video based motion analysis of ultrasound probe movement and heel displacement measurements (Fig. 10). Ultrasound images of MG MTJ were taken at 70 Hz using a 6 cm linear array probe (UST-5712, Aloka, Japan). The probe was positioned over the GM MTJ, approximately two centimeters medial to the border between medial and lateral gastrocnemius, and secured with an elastic band. MTJ location in the ultrasound image coordinate system was measured using custom software (Magnusson et al. 2003b) utilizing Lukas-Kanade feature tracking (Bouguet 2001). The software tracked movement of nine points that were placed along the aponeurosis between GM and SOL, just proximal to the GM MTJ to reflect movement of the MTJ. Each file was analyzed twice and the average was used for subsequent analysis. Ultrasound probe location in 2D was measured from video taken in the sagittal plane (InLine 250, Fastec Imaging, USA at 60 Hz) using Peak Motus 2000 software (Peak Performance Technologies, USA). This information was used to transform GM MTJ location to the laboratory coordinate system. The location of the Achilles tendon insertion in the laboratory coordinate system was measured using a potentiometer located under the heel. Finally, Achilles tendon length was defined as the distance between Achilles tendon origin and insertion and Achilles tendon elongation calculate by subtracting tendon length at rest from tendon length during the contractions.

The repeated contractions were first separated. Then tendon force and elongation data were time normalized and averaged to produce one force-elongation relationship for each subject. Tendon stiffness and Young's modulus were calculated between 10% and 80% of MVC force as the slope of the linear fit to tendon force-elongation and stress-strain relationships, respectively. Within this region the relationships were highly linear (Fig. 10). Tendon hysteresis was calculated as the percentage difference in the area under the curve for the loading and unloading phases of the curve.

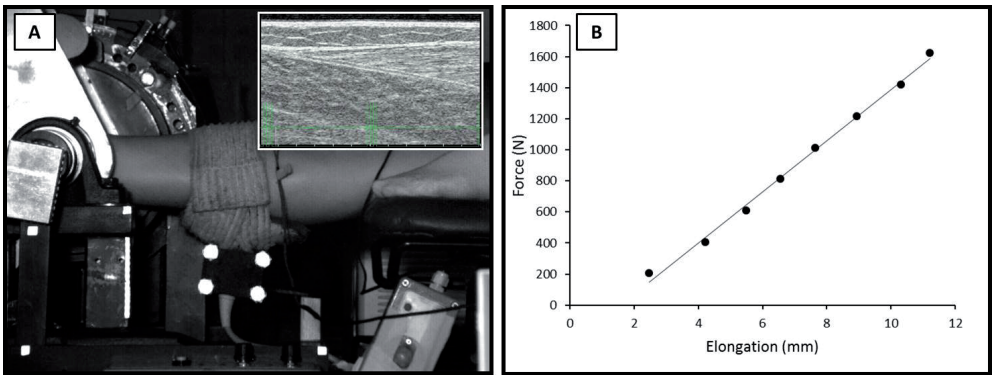

FIGURE 10 Measurement of Achilles tendon mechanical properties. (A) Isometric plantarflexion contractions were performed in a dynamometer with synchronous measurement of pedal reaction force, heel displacement, ultrasound probe location and MG MTJ displacement (inset shows ultrasound image of the MTJ). (B) Average Achilles tendon force-elongation plot from 10 to 80% MVC at 10% MVC increments from one subject. Achilles tendon stiffness was determined as the slope of a linear fit to the force-elongation data.

4.2.5 Physical function in older adults (II)

Physical function was assessed by two walking tests; the 6-minute walk test (6MWT) and timed "up and go"-test (TUG). Both tests are widely used in aging research and have been shown to have good test-retest reliability (Harada et al. 1999, Payette et al. 1998).

The 6MWT was performed on a 20 m course marked with cones at both ends. Subjects were instructed to walk the longest distance possible during 6 minutes without running (Enright 2003). Subjects were informed at the end of each minute about the time elapsed and were given standardized encouragement. The total distance covered during 6 minutes was taken as the final result.

In the TUG-test, subjects were asked to stand up when hearing the "go" command and walk around a cone placed 3 meters away and sit back down on the seat (Podsiadlo & Richardson 1991). The task was performed as fast as possible. Time taken to perform the task was measured using a stopwatch. The task was performed three times and the fastest performance was taken as the final result.

4.2.6 Lower extremity lean mass (II)

Lower extremity lean mass (excluding bone mass) was measured using dual-energy X-ray absorptiometry (Lunar Prodigy, version EnCore 9.30) while wearing only light clothes. Software preset regions for left and right legs were used and lean mass of the legs was summed to attain lower extremity lean mass in kilograms.

4.2.7 Knee extension strength (II)

Knee extension strength was measured in a custom-built dynamometer (University of Jyväskylä, Finland) with maximal voluntary isometric knee extension. Subjects sat in the dynamometer with the knee and hip flexed at 90° angles and waist and ankle tightly strapped to the dynamometer. The tested leg was the leg that the subject self-reported as the strongest. After a standardized warm-up, three maximal voluntary contractions were performed. The force measured by the force transducer was converted to knee joint torque by multiplying the measured force by the distance from the knee joint center to the force transducer (measured using a measuring tape). The highest torque value was used in subsequent analysis.

4.2.8 Leg extension power (II)

Leg extension power was measured during maximal countermovement jumps performed on a force platform (custom-built force platform, University of Jyväskylä, Finland). Subjects were instructed to jump as high as possible while keeping their hands on their hips. Power was calculated from the measured ground reaction force data using the method described by Caserotti and Simonsen (2001). Briefly, center of mass vertical velocity was derived by means of numerical integration of center of mass vertical acceleration, and instantaneous power was calculated as the product of vertical velocity and vertical ground reaction force. Peak power in the concentric phase of the jump was taken as leg extension power. Three maximal jumps were performed and the highest power value was used in subsequent analysis.

4.2.9 Physical activity levels (II)

Habitual physical activity level of older adults was assessed using the Voorrips physical activity questionnaire (Voorrips et al. 1991). The questionnaire is designed for older adults and covers household, sport and leisure time activities. The total score of the questionnaire was used as a measure of the level of habitual physical activity.

4.2.10 Triceps surae muscle-tendon function during walking (IV)

Subjects walked over a 10-meter force platform at their preferred walking speed. They started walking three meters before and stopped walking three meters after the force platform to ensure that walking speed was stable over the force platform. The trials were timed using photocells at the start and end of the force platform and the average walking speed was calculated from the measured time. The average walking speed of three consecutive trials with walking speeds within ±5% was defined as preferred walking speed. In addition to the preferred walking speed, older subjects performed trials at a speed that matched the mean preferred speed of young subjects within ±5%. In both preferred and matched speed trials, data were recorded from at least three trials. In

addition, all subjects performed one trial with maximal walking speed from which only the time of the trial was recorded.

During walking, three-dimensional GRFs were measured using a 10-meter long custom-built force platform (University of Jyväskylä, Finland). Two force platforms parallel to each other enabled measurement of GRFs separately for both legs. GRFs were low-pass filtered at 40 Hz using a fourth order zero-lag Butterworth filter and normalized by dividing the forces by body weight.

An ultrasound probe (7.5 MHz linear array probe with 60 mm field width, Telemed, EchoBlaster128, Lithuania) was attached over the MG muscle visualizing both MG and soleus simultaneously. Ultrasound images were collected at 80 Hz during walking. Muscle fascicle lengths and pennation angles of MG and soleus were tracked from the ultrasound images using an automatic tracking algorithm (Cronin et al. 2011) and low-pass filtered at 40 Hz using a fourth order zero-lag Butterworth filter. Muscle fascicle lengths obtained from walking trials were normalized by dividing them by muscle fascicle reference lengths. The reference lengths were measured in a resting condition while subjects were seated in a dynamometer with the knee fully extended and ankle angle at 90°.

Sagittal plane ankle and knee joint kinematics were measured using custom built electrogoniometers (University of Jyväskylä, Finland) attached over the lateral side of the joint. MTU lengths were calculated from the joint kinematics using regression equations defined by Hawkins and Hull (1990). TT length was calculated as $L_{TT} = L_{MTU} - L_{FAS}*\cos(\text{pennation angle})$, where L_{MTU} is MTU length and L_{FAS} is muscle fascicle length (Fukunaga et al. 2001). MTU and TT lengths were normalized by dividing them by their corresponding reference lengths determined in the same way as muscle fascicle reference lengths.

Strides were separated based on vertical GRF, interpolated to 1000 data points and averaged across subject and speed (including ground reaction forces and muscle activity). The start of the push-off phase was identified from the anterior-posterior GRF. Since TT and muscle fascicles are connected in series, peak fascicle force generation was estimated to occur at the instant of peak TT length.

To determine muscle activity in walking, electromyography (EMG) was measured from MG and soleus using a telemetric EMG system (Noraxon Inc. Scottsdale, AZ, USA). After shaving and preparing the skin with fine sandpaper and alcohol wipes, two electrodes (Ambu A/S, Ballerup, Denmark) were attached over the skin with 22 mm interelectrode distance according to SENIAM recommendations (Hermens et al. 1999). The data were sampled at 1500 Hz, preamplified and sent wirelessly to an A/D converter (Cambridge Electronic Design, Cambridge, UK).

EMG data were band-pass filtered between 20 and 450 Hz using a fourth order zero-lag Butterworth filter. Root mean squared (RMS) values during stance and push-off phases were calculated from each stride and normalized to maximal 500 ms RMS obtained during MVIC tests. RMS values were then averaged across subject and speed. For visualization, moving 100 ms RMS windows were used to produce EMG envelopes.

4.3 Statistical analyses

There were some missing data due to technical problems or due to inadequate image quality of ultrasound images. Soleus muscle architecture data were missing from three older men (I and III) and five older women (I). Regarding triceps surae muscle function during walking (IV), soleus muscle fascicle data from one older subject were not analyzed due to inadequate image quality, and EMG data was lost from one subject due to technical problems.

All data are reported as mean ± standard deviation. Normality of the data was tested using Shapiro-Wilk test, and homogeneity of variances using Levene's test. The level of statistical significance for all tests was set at $p<0.05$. Statistical analyses were performed using IBM SPSS Statistics software (SPSS, Chicago, Illinois, USA).

Differences between groups (age groups and training backgrounds) were tested using analysis of variance and analysis of covariance. Analysis of covariance was used to statistically adjust the results for tibia lengths in the case of muscle fascicle lengths, and for tendon loading in the case of tendon mechanical properties. Bonferroni correction was applied to pairwise comparisons of the groups. Correlation between Achilles tendon mechanical properties and Achilles tendon loading was tested using Pearson's product-moment correlation.

Two-tailed independent samples Student's t-test was used to test differences between young and older adults in muscle function during walking. Differences between preferred and matched speed within older adults were tested using paired samples t-test.

To examine the association between physical function (6MWT and TUG) and lower limb muscle-tendon properties in older adults, regression modeling was performed. Only the subjects with a complete dataset were selected for these analyses, resulting in a sample of 26 older women and 26 older men. First, possible covariates for physical function (anthropometrics, age, sex and habitual physical activity level) were tested using Pearson's product-moment correlation and the factors correlating with physical function (age, sex, body mass and height) were used as adjusting factors in subsequent partial correlations and multivariate regression models. After partial correlations, multivariate modeling was performed to determine the independent effects of the factors that showed significant partial correlations with physical function. For regression analyses, muscle fascicle lengths were normalized to body stature by dividing fascicle length by tibia length.

5 RESULTS

This chapter presents the main results of the thesis. For more details the original papers (I-IV) should be consulted.

5.1 Age- and training-related differences in plantarflexion muscle strength

When normalized to body weight, older adults had 39% lower plantarflexion strength compared to young adults (1.25 vs. 2.04 body weights, $p<0.001$). There was no significant age*sex interaction.

When comparing young and older untrained adults to older athletes, it was observed that older endurance runners had 37% lower (1.31 vs. 2.08 body weights, $p<0.001$) and older sprint runners 20% lower (1.67 vs. 2.08 body weights, $p=0.029$) plantarflexion strength compared to young adults. No significant differences were observed between older athletes and untrained older adults ($p>0.05$).

5.2 Age- and training-related differences in triceps surae muscle architecture

Mean values of triceps surae muscle architectural parameters in each subject group are summarized in table 4. Significant age*sex interactions were not observed for any muscle architectural parameter analyzed. For sex differences readers are referred to paper I.

5.2.1 Age-related differences in triceps surae muscle architecture

Older adults had significantly shorter MG fascicle length compared to young adults (p=0.022) with an adjusted marginal mean difference of 3.6 mm (8%). No significant differences between young and older adults were observed in soleus fascicle length (p=0.477) or pennation angles in MG (p=0.250) or soleus (p=0.116).

Older adults had smaller triceps surae muscles. MG thickness was smaller by 2.5 mm (p<0.001, 13%) and soleus by 1.4 mm (p=0.032, 10%). Combined anatomical CSA of medial and lateral gastrocnemius was 3.3 cm^2 smaller in older compared to young adults (p=0.001, 15%).

5.2.2 Training-related differences in triceps surae muscle architecture

Muscle architectural parameters measured from older athletes were compared to values from untrained young and older adults. It was observed that older endurance runners had shorter soleus fascicle length compared to both young (p=0.009, 26%) and older untrained adults (p=0.033, 22%). No significant differences were observed between the groups regarding soleus pennation angle and muscle thickness or MG fascicle length, pennation angle or thickness (p>0.05). Older sprint runners had 20% greater combined anatomical CSA of MG and LG compared to untrained older adults (p=0.013).

TABLE 4 Triceps surae muscle architectural parameters separated by subject groups.

	Young		Older		Older athletes	
	Women	Men	Women	Men	Endur.	Sprint
MG fascicle length (mm)	47.3±6.7	47.7±6.6	42.7±6.6	45.0±7.6	45.3±6.9	47.7±7.0
MG pennation angle (deg)	24.7±4.0	24.8±4.0	23.2±3.7	24.4±4.2	23.3±4.8	24.1±3.5
MG thickness (mm)	19.2±2.7	20.1±2.5	16.6±2.0	17.7±3.2	17.2±3.6	18.6±2.7
Soleus fascicle length (mm)	41.6±11.1	40.6±8.8	41.0±8.3	38.6±7.6	31.2±3.9	35.3±8.3
Soleus pennation angle (deg)	19.5±5.1	21.0±5.7	16.2±3.2	21.2±4.0	23.7±5.3	21.6±8.3
Soleus thickness (mm)	13.4±2.6	14.3±2.6	11.9±3.3	13.1±2.7	13.4±2.7	12.8±3.7
Gastrocnemius CSA (cm^2)	20.5±4.0	24.2±4.5	18.2±4.2	20.1±4.5	20.9±3.4	25.1±4.4

Values are presented as mean ± standard deviation.

5.3 Age- and training-related differences in Achilles tendon properties

Mean values of Achilles tendon properties in each subject group are summarized in table 5. Significant age*sex interactions were not observed for any Achilles tendon parameter analyzed. For sex differences readers are referred to paper I.

5.3.1 Age-related differences in Achilles tendon properties

Achilles tendon CSA was 17% larger in older compared to young adults (62.1 vs. 53.2 mm², p<0.001). Achilles tendon stiffness was 16% lower in older compared to young adults (142 vs. 169 N/mm, p=0.002). However, after adjusting for tendon force during MVIC, no significant difference was observed between the age groups (153 vs. 146 N/mm, p=0.517). This is illustrated in Fig. 11 showing the relationship between tendon force at MVIC vs. stiffness. No apparent difference in this relationship is observable between young and older adults. Young's modulus was 32% lower in older compared to young adults (0.54 vs. 0.79 GPa, p<0.001). Adjustment for tendon stress at MVIC removed the age-related difference in Young's modulus (0.61 vs. 0.63 GPa, p=0.674). No significant difference between the age groups was observed in hysteresis (2.5 vs. 3.2%, p=0.548). The combination of lower plantarflexion strength and greater Achilles tendon CSA in older adults led to 49% lower Achilles tendon stress at MVIC in older compared to young adults (p<0.001). Strain at 80% of MVIC force was 20% lower in older compared to young adults (4.8 vs. 6.0%, p<0.001).

TABLE 5 Achilles tendon properties separated by subject groups.

	Young		Older		Older athletes	
	Women	Men	Women	Men	Endur.	Sprint
Cross-sectional area (mm²)	49.8±8.8	56.5±9.6	55.2±8.8	69.0±12.2	82.0±19.9	96.5±24.9
Stiffness (N/mm)	151±29	186±37	120±39	164±47	172±39	166±35
Young's modulus (GPa)	0.71±0.18	0.86±0.2	0.48±0.18	0.59±0.17	0.56±0.22	0.48±0.19
Hysteresis (%)	5.0±6.0	1.4±3.7	2.3±5.7	2.8±4.4	2.0±14.2	2.4±11.8
Stress at MVIC (MPa)	55.3±15.2	60.0±14.3	26.3±9.0	33.1±9.0	25.3±6.8	28.5±13
Strain at 80% MVIC (%)	6.4±1.5	5.7±1.5	4.9±1.7	4.8±1.2	4.5±1.8	4.7±1.7

Values are presented as mean ± standard deviation.

5.3.2 Training-related differences in Achilles tendon properties

Comparisons of older athletes to untrained young and older adults did not show significant differences between the groups in Achilles tendon stiffness, hysteresis or strain at 80% MVIC force ($p>0.05$). Tendon CSA in older sprint runners was 71% greater compared to untrained young adults ($p<0.001$) and 40% greater compared to untrained older adults ($p<0.001$) but a significant difference was not observed compared to older endurance runners ($p=0.216$). Tendon CSA in older endurance runners was 45% greater compared to untrained young adults ($p<0.001$). All older groups had significantly lower Young's modulus compared to untrained young adults ($p\leq0.001$) without any significant differences between the groups of older adults ($p>0.05$). No significant differences in Young's modulus were observed between the groups after adjusting for tendon stress at MVIC ($p=0.440$). Tendon stress at MVIC was significantly greater in untrained young adults compared to all groups of older adults ($p<0.001$) without any significant differences between groups of older adults ($p>0.05$).

5.3.3 Association between Achilles tendon mechanical properties and tendon loading

A significant positive correlation was observed between Achilles tendon stiffness and Young's modulus and measures of tendon loading. These relationships are presented in figure 11. As reported in previous chapters, no significant differences were observed in these relationships between young and older adults. Tendon force at MVIC explained 36% of the variance in tendon stiffness and tendon stress at MVIC explained 51% of the variance in tendon Young's modulus.

Although not the main interest of the current thesis, it is noteworthy that there was a significant main effect of sex on these relationships. On average, women had 19 N/mm lower tendon stiffness and 0.07 GPa lower tendon Young's modulus at a given level of tendon force and stress at MVIC, respectively.

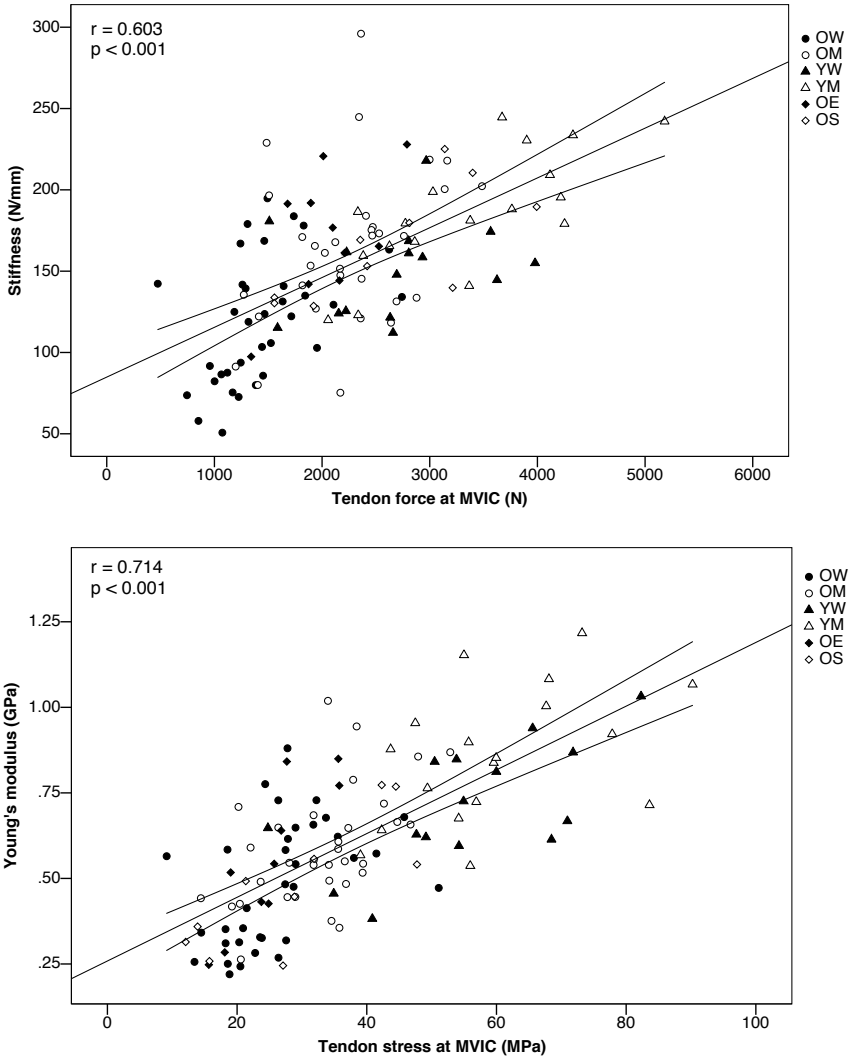

FIGURE 11 Relationships between tendon stiffness and tendon force at MVIC (upper) and between tendon Young's modulus and tendon stress at MVIC (lower). Lines represent linear least squares fits and 95% confidence intervals. OW older women, OM older men, YW young women, YM young men, OE older endurance runners and OS older sprint runners.

5.4 Associations between physical function and triceps surae muscle-tendon properties in older adults

Partial correlations between physical function (6MWT and TUG) and selected muscle-tendon properties, adjusted for age, sex, body mass and height, are re-

ported in table 6. Longer distance walked in 6MWT was associated with greater plantarflexion strength, Achilles tendon stiffness, soleus pennation angle, knee extension strength, leg extension power and shorter medial gastrocnemius and soleus fascicle lengths ($p<0.05$). Shorter TUG time was associated with greater plantarflexion strength, Achilles tendon stiffness, soleus pennation angle, leg extension power and shorter soleus fascicle length ($p<0.05$).

Multivariate regression models, adjusted for age, sex, body mass and height, were performed including the muscle-tendon parameters that were associated with 6MWT and TUG based on partial correlation. The results of the multivariate models are presented in table 7.

The multivariate model predicted 73% (based on R^2, see table 7 for adjusted R^2) of the variance in 6MWT distance. In this model, plantarflexion strength, Achilles tendon stiffness and medial gastrocnemius fascicle length were independent predictors of 6MWT performance. The multivariate model predicted 61% of the variance in TUG time. Independent predictors in this model were plantarflexion strength and soleus fascicle length.

TABLE 6 Partial correlations between physical function and lower limb muscle-tendon properties.

	6MWT (m)		TUG (s)	
	Partial r	P-value	Partial r	P-value
Plantarflexion strength (N)	**0.482**	**0.001**	**-0.434**	**0.002**
Achilles tendon stiffness (N/mm)	**0.519**	**<0.001**	**-0.381**	**0.007**
MG fascicle length (mm/mm)	**-0.287**	**0.048**	0.093	0.528
MG pennation angle (°)	0.188	0.603	0.049	0.742
MG thickness (mm)	-0.077	0.603	0.013	0.931
Soleus fascicle length (mm/mm)	**-0.302**	**0.037**	**0.291**	**0.045**
Soleus pennation angle (°)	**0.422**	**0.003**	**-0.298**	**0.040**
Soleus thickness (mm)	0.073	0.622	-0.053	0.719
Lower extremity lean mass (kg)	0.155	0.292	-0.125	0.396
Knee extension strength (Nm)	**0.360**	**0.012**	-0.282	0.053
Leg extension power (W)	**0.443**	**0.002**	**-0.419**	**0.003**

Adjusted for age, sex, body mass and height. Statistically significant associations are bolded.

TABLE 7 Multivariate regression models with 6MWT and TUG as dependent variables and lower limb muscle-tendon properties as independent variables.

	Adj. R^2	Stand. β	P-value
6MWT (m)	0.651		
Plantarflexion strength (N)		**0.340**	**0.022**
Achilles tendon stiffness (N/mm)		**0.272**	**0.020**
MG fascicle length (mm/mm)		**-0.208**	**0.046**
Soleus fascicle length (mm/mm)		-0.121	0.269
Soleus pennation angle (°)		0.110	0.358
Knee extension strength (Nm)		0.038	0.831
Leg extension power (W)		0.321	0.098
TUG (s)	0.530		
Plantarflexion strength (N)		**-0.335**	**0.037**
Achilles tendon stiffness (N/mm)		-0.169	0.193
Soleus fascicle length (mm/mm)		**0.245**	**0.031**
Soleus pennation angle (°)		-0.027	0.841
Leg extension power (W)		-0.363	0.096

Adjusted for age, sex, body mass and height. P-values for the models <0.001.

5.5 Triceps surae muscle-tendon function during walking

Mean muscle fascicle length and velocity, TT and MTU lengths and EMG patterns of MG and soleus during walking across the stride cycle are presented in figure 12.

5.5.1 Spatio-temporal gait parameters

Preferred walking speed was 18% slower in older compared to young adults (1.11±0.12 vs. 1.35±0.16, p<0.001). Naturally, matched speed was similar between the groups (p=0.946). At preferred speed, older adults walked with lower stride frequency (p=0.003) and length (p=0.002) and with greater duty factor (i.e. relative duration of stance to stride, p=0.024). At matched speed there were no significant differences between the groups in the aforementioned spatio-temporal gait parameters. Maximal walking speed was 13% slower in older compared to younger adults (2.01±0.22 vs. 2.31±0.39 m/s, p=0.024).

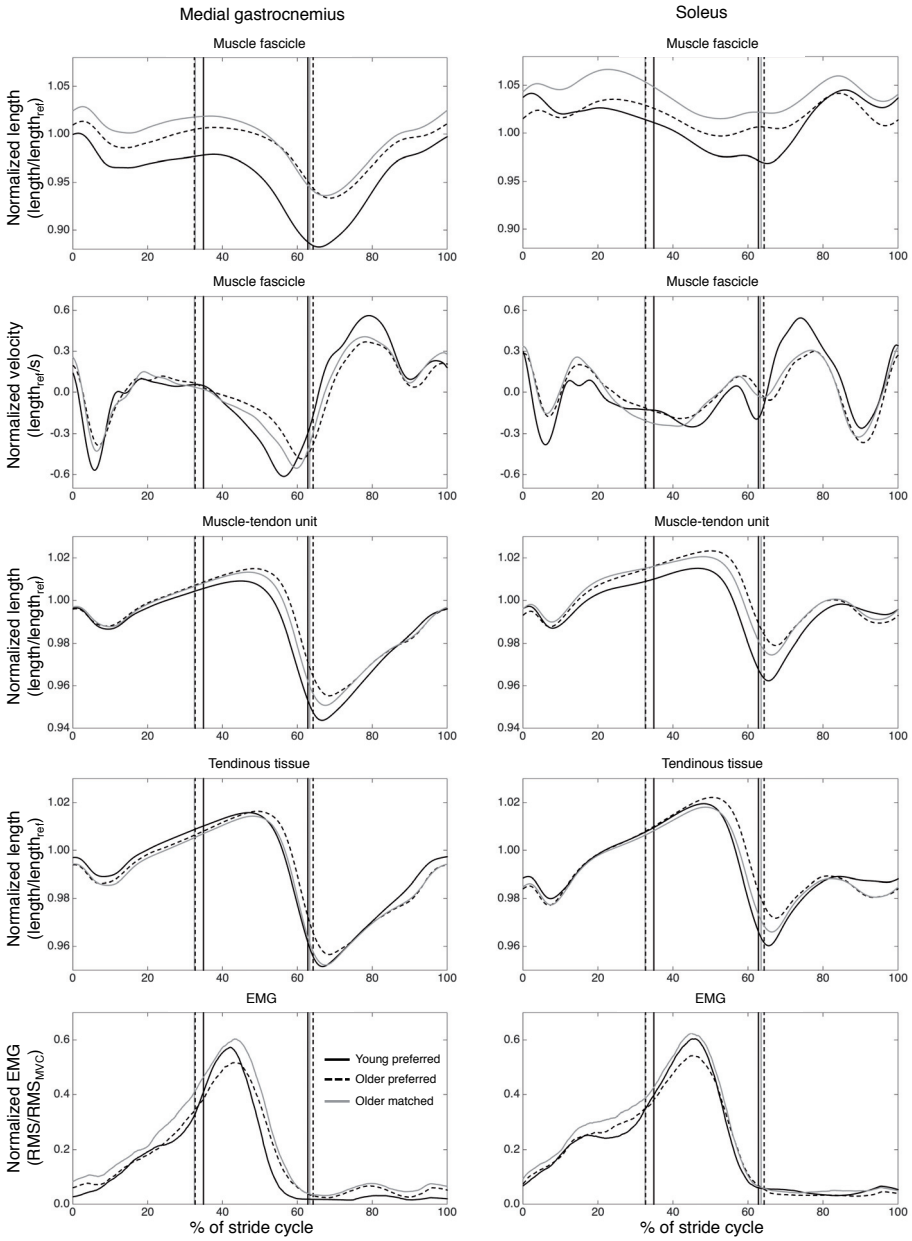

FIGURE 12 Group mean normalized muscle fascicle length and velocity, muscle-tendon unit and tendinous tissue lengths and EMG data. The left column shows results from medial gastrocnemius and the right column from soleus. Vertical lines represent the start of the push-off phase and toe-off respectively. Standard deviations are omitted for clarity.

5.5.2 Muscle fascicle lengths

During the stance phase, older adults utilized a narrower operating range in both MG and soleus compared to young adults at preferred speed (Fig. 13, MG 7.5±2.7 vs. 12.0±4.8% of reference length, p=0.014, soleus 4.9±1.7 vs. 8.5±3.9% of reference length, p=0.007). No significant differences were observed when the groups were compared at matched speed. In older adults, increase in walking speed significantly increased the operating range in both MG (7.5±2.7 vs. 9.3±3.1% of reference length, p=0.003) and soleus (4.9±1.7 vs. 6.5±1.7% of reference length, p<0.001).

There were no differences between young and older adults in the lengths at which muscle fascicles operated, since no significant differences were observed in normalized mean fascicle lengths calculated from the whole stance phase or the push-off phase, or in fascicle length at peak TT length (p>0.05).

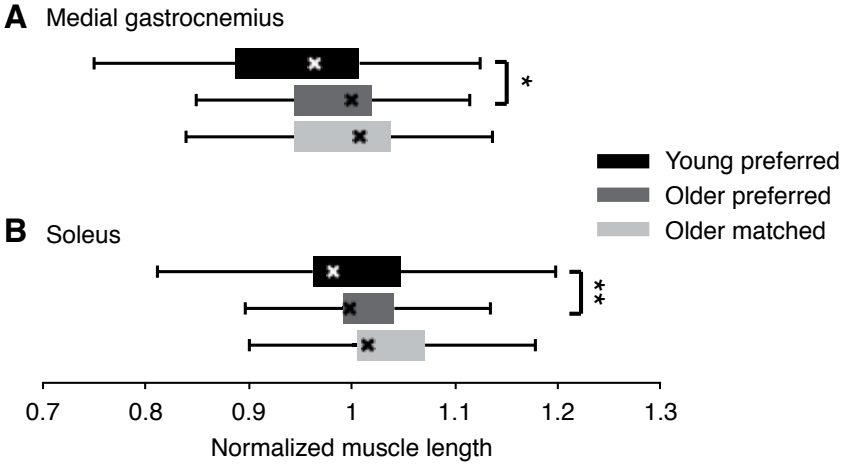

FIGURE 13 Normalized medial gastrocnemius (A) and soleus (B) fascicle operating lengths during the stance phase. Whiskers represent standard deviations of lower and upper limit. Crosses mark fascicle lengths at peak tendinous tissue elongation. Fascicle lengths are normalized to resting length at 90° ankle angle and knee extended. * p<0.05, ** p<0.01.

5.5.3 Muscle-tendon unit and tendinous tissues lengths

MTU peak length and length at toe-off were significantly longer in older compared to young adults in both muscles at preferred speed (peak length: MG p=0.044, soleus p=0.022, length at toe-off: MG p=0.032, soleus p=0.021), but the range during stance did not differ between the groups (p>0.05). At matched speed, no significant differences were observed between young and older adults in MTU lengths or ranges. MG MTU range significantly increased in older adults when walking speed was increased from preferred to matched (p=0.043).

TT maximal elongation and strain did not differ between the groups, nor did they change with speed in older adults. Pooling the data from both groups and both speeds of older adults resulted in the following mean TT maximal elongation and strain values: MG 27.2±5.3 mm and 6.4±1.2%, soleus 16.3±4.7 mm and 5.9±1.7%.

5.5.4 Muscle-tendon unit and fascicle shortening velocities

Significant differences were not observed in MTU or fascicle peak shortening velocities between the age groups. Soleus peak shortening velocity during push-off was significantly higher at the faster walking speed in older adults ($p=0.010$) and a similar tendency was observed in MG ($p=0.066$). Fascicle shortening velocity at peak TT length was significantly lower in older compared to young adults at preferred speed (MG $p=0.048$, soleus $p=0.020$) but not at matched speed (MG $p=0.545$, soleus $p=0.208$). Soleus fascicle shortening velocity at peak TT length was significantly higher at the faster walking speed in older adults ($p=0.004$) but a statistically significant difference was not observed in MG ($p=0.094$).

5.5.5 Muscle-tendon interaction

In both MG and soleus, muscle fascicle length changes were clearly decoupled from the length changes of the MTU. Contributions of muscle fascicle to MTU length changes during stance were significantly lower in older compared to young adults at preferred speed in both muscles (MG 0.26±0.10 vs. 0.16±0.05, $p=0.012$, soleus 0.23±0.08 vs. 0.15±0.04, $p<0.001$, Fig 14 A). Significant differences were not observed when young and older adults were compared at matched speeds. Increase in walking speed significantly increased muscle fascicle to MTU length change ratio in older adults (MG 0.16±0.05 vs. 0.19±0.07, $p=0.013$, soleus 0.15±0.04 vs. 0.21±0.08, $p=0.002$).

Muscle fascicle velocities were clearly different from MTU velocities. Muscle fascicle relative to MTU peak shortening velocities during push-off did not significantly differ between young and older adults or within older adults between different walking speeds in either muscle (Fig. 14 B). Pooling both groups and speeds together, MTU peak shortening velocities during push-off were on average 8 and 11 times greater than peak shortening velocity of the muscle fascicles in MG and soleus, respectively.

5.5.6 Electromyography

Normalized RMS EMG activity of MG and soleus did not significantly differ between young and older adults at either walking speed when compared during stance or push-off. However, the EMG activity significantly increased in both stance and push-off in both muscles in older adults as they increased their walking speed from preferred to matched speed ($p<0.026$).

5.5.7 Ground reaction forces

Peak propulsive and resultant forces during push-off significantly increased in older adults when changing walking speed from preferred to matched (propulsive 0.16±0.03 vs. 0.19±0.03 body weights, $p<0.001$, resultant 1.03±0.07 vs. 1.05±0.08 body weights, $p=0.012$). However, both peak propulsive and resultant forces during push-off remained significantly lower in older compared to young adults at matched speed (propulsive 0.19±0.03 vs. 0.22±0.04 body weights, $p=0.011$, resultant 1.05±0.08 vs. 1.14±0.08 body weights, $p=0.045$).

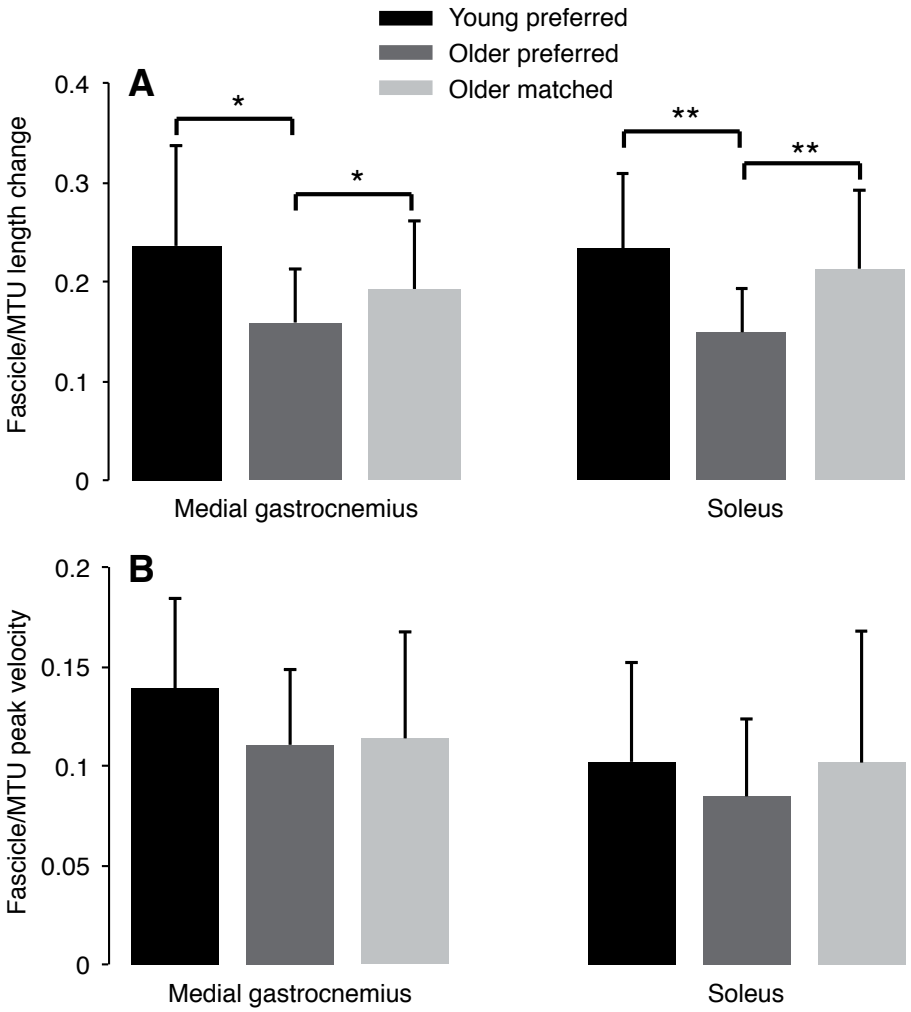

FIGURE 14 Ratios of muscle fascicle to muscle-tendon unit length change during stance (A) and peak shortening velocity during push-off (B). * $p<0.05$, ** $p<0.01$.

6 DISCUSSION

The main findings of the current thesis were:

1) Older compared to young adults had shorter MG fascicle length and smaller triceps surae muscle size. Achilles tendon stiffness and Young's modulus were lower but Achilles tendon CSA was larger in older compared to young adults. Achilles tendon stiffness and Young's modulus were related to loading of the tendon during maximal isometric plantarflexion, but there were no significant age-related differences in the relationships (I).

2) Older adults trained for sprint running had larger Achilles tendon CSA and older adults trained for endurance running had shorter soleus fascicle length compared to both young and older untrained adults. In addition, sprint trained older adults had larger gastrocnemius anatomical CSA compared to untrained older adults (III).

3) Triceps surae muscle-tendon properties were associated with physical function in older adults. Specifically, greater plantarflexion strength, shorter MG fascicle length and greater Achilles tendon stiffness were associated with longer distance walked in 6MWT. Moreover, greater plantarflexion strength and shorter soleus fascicle length were associated with shorter TUG time independent of lower extremity muscle mass, knee extension strength and leg extension power (II).

4) Triceps surae muscle-tendon function during walking was dependent on walking speed, with no significant age-related differences between young and older adults when the groups were compared at matched walking speed. When the groups were compared at preferred walking speeds, older adults showed a narrower fascicle operating range, lower shortening velocity at esti-

mated peak force generation and a greater relative contribution of TT to MTU length changes (IV).

6.1 Age-related differences in triceps surae muscle architecture

Comparison of untrained young and older adults revealed that MG fascicle length was 8% shorter in older compared to young adults, without significant differences in MG or soleus pennation angles or soleus fascicle length. Age-related differences in triceps surae muscle thickness and gastrocnemius anatomical CSA were more consistent, with 10% to 15% lower values in older compared to young adults. Plantarflexion strength was 37% lower in older compared to young adults. These results suggest a loss of muscle mass, both in series and in parallel, and loss of muscle quality, i.e. loss of force generation capacity per PCSA since age-related difference in plantarflexion strength was markedly greater compared to age-related differences in the measures of triceps surae muscle size. These findings corroborate previous more direct measurements of muscle quality in aging (Morse et al. 2005c).

Only a few differences in muscle architecture were observed between older athletes and untrained older or young adults. Older endurance runners had shorter soleus muscle fascicle length compared to young and older untrained adults. It may be that this is an endurance training-induced adaptation that improves efficiency of running. Soleus acts as a force rather than a power generator in locomotion, thus shorter muscle fascicles may reduce the energy cost of force generation by reducing the amount of activated muscle volume for a given force output (Biewener & Roberts 2000). Additionally, the results may reflect greater loss of sarcomeres in series than in parallel in the endurance trained older adults in order to preserve muscle PCSA. However, it should be acknowledged that fascicle length measurements using ultrasound imaging, like done in the current thesis, do not provide definite indication of number of in series sarcomeres since it is possible that the fascicles length are measured at different sarcomere lengths i.e. at different length relative to the optimum length.

Older sprint-trained adults had 20% larger gastrocnemius muscle anatomical CSA compared to untrained older adults. This finding is supported by previous observations in young adults showing that sprint running but not endurance running is associated with greater muscle strength and size of plantarflexor muscles (Hansen et al. 2003, Kubo et al. 2011). It may be that sprint training preferentially targets gastrocnemius muscle, which contains more fast-twitch muscle fibers than soleus (Edgerton et al. 1975), and this may be the reason why greater muscle thickness was not observed in soleus in sprint trained compared to untrained older adults.

Greater loss of muscle power compared to muscle strength has been observed in older adults (Raj et al. 2010). This could be partly due to a decrease in muscle fascicle length, resulting in greater sarcomere shortening velocity at a given joint angular velocity and hence lower force generation due to the force-velocity relationship. However, in triceps surae muscles, it may be preferable to

lose sarcomeres in series by reducing fascicle length rather than a similar loss of muscle mass via a reduction in sarcomeres in parallel. The reason for this is that the triceps surae muscle fascicles show relatively slow shortening velocities during walking and running (Farris & Sawicki 2012), and thus function as force rather than power generators. Thus, the shorter fascicle lengths observed in untrained older compared to young adults in MG, and in endurance trained older adults compared to untrained young and older adults in soleus, may be an adaptation that serves to preserve PCSA with aging. In older endurance runners, this adaptation may be observed in soleus because of the important role that soleus has in endurance performance due to its high proportion of type I muscle fibers (Edgerton et al. 1975). As stated above, ultrasound imaging based measurements of fascicle length do not provide valid indication of the number of sarcomeres in series. Therefore, direct invasive measurement of sarcomere length or alternatively measurement of optimum fascicle length (i.e. the length at which sarcomeres are at their optimum length) would be required to validate the hypotheses presented in this chapter.

Collectively, the findings regarding muscle architectural measurements taken at rest suggest that aging has only a modest effect on muscle architecture in triceps surae. Shorter muscle fascicle lengths observed in older compared to young adults may contribute to the previously observed greater loss of muscle power compared to muscle strength (Raj et al. 2010), but may also provide a mechanism to preserve muscle PCSA in spite of loss of muscle mass. Furthermore, the present results suggest that sprint running but not endurance running may help to mitigate age-related loss of muscle mass in the triceps surae muscles.

6.2 Age-related differences in Achilles tendon properties

Achilles tendon CSA in untrained older adults was 20% larger compared to untrained young adults. In addition, older sprint runners had 71% larger Achilles tendon CSA compared to untrained young adults and 40% larger compared to untrained older adults. Endurance trained older adults had 45% larger CSA compared to untrained young adults. These findings give rise to the idea that Achilles tendon responds to regular loading by increasing CSA in an intensity-dependent manner, and that this effect is additive to the increase due to normal aging. This idea is supported by the findings of Couppé et al., (2014) showing that regular endurance running was associated with larger patella tendon CSA in both young and older adults.

It is currently unknown why greater CSA is observed in older compared to young adults. One explanation could be that a compensatory increase in CSA is needed to maintain appropriate tendon mechanical properties (stiffness) in relation to muscle force generation capacity, as tendon material properties decline with aging. An increase in CSA would also reduce tendon stress for a given force level, which may be important for reducing injury risk, since older tendons may have a reduced ultimate tensile stress. This hypothesis fits well with

the observation that the difference in Young's modulus between young and older adults was approximately double the difference in stiffness. An alternative explanation of the current observations could be that tendon material properties are maintained relatively unaltered with aging. However, tendon CSA may increase due to an increase in intratendinous water or fat (Finlayson & Woods 1975), which would increase tendon CSA without markedly altering stiffness. As a consequence, a decrease in Young's modulus would be observed. Support for this hypothesis comes from a study by Heinemeier et al. in which it was observed that collagen matrix is hardly renewed after age 17, at least in the core of the Achilles tendon (Heinemeier et al. 2013). The mechanism responsible for greater tendon CSA in older adults remains elusive, but it seems that it is not linked to reduced physical loading of the tendon with aging, as older athletes showed even greater tendon CSA compared to untrained older adults. Additionally the data suggest that the effects of aging and physical loading on tendon CSA could be additive with inducing increase in tendon CSA. However, mechanisms behind these effects could be different.

Lower Achilles tendon stiffness and Young's modulus were observed in older compared to young adults, but statistical adjustment of Achilles tendon stiffness for maximal tendon force during MVIC and Young's modulus for maximal stress during MVIC removed the differences between the age groups. Additionally it was observed that stiffness and Young's modulus were linearly related to maximal tendon force and stress, respectively. These findings suggest that tendon mechanical properties adapt to physical loading. More specifically, the findings suggest that tendon elongation or strain is a variable that is similar irrespective of age. The functional relevance of this could be to limit maximal strain, thereby preventing strain-induced tendon damage, or to maintain optimal muscle-tendon interaction with changes in muscle strength. However, although a linear relationship between tendon loading and tendon mechanical properties was observed, there was a nonzero intercept of the regression line (Fig. 11) meaning that tendon force and stress were not proportionally related to tendon mechanical properties. As a consequence, tendon elongation and strain at a given relative load, such as 80% of MVIC, differs between individuals with different muscle strengths. Hence, older adults, who generally have lower muscle strength, also have lower maximal tendon elongation and strain on average. Estimation of tendon elastic energy storage based on tendon stiffness and tendon force at MVIC yields about 50% lower maximal elastic energy storage in older compared to young adults. This may have functional implications for locomotion performance and efficiency. The mechanisms responsible for adjusting tendon mechanical properties to match muscle force generation capacity are unknown and may change with aging. Aging is associated with an increase in advanced glycation end-product cross-links in tendon collagen matrix (Couppé et al. 2009, Couppé et al. 2014), which may increase tendon stiffness. However, there may be effects that counteract the effect of increased collagen cross-links on tendon stiffness, to allow stiffness to be related to muscle strength without a significant effect of age. Structural differences in tendons of different age but similar whole tendon stiffness may be a factor contributing to the greater inci-

dence of tendon ruptures in middle-aged compared to young adults (Lantto et al. 2015).

The mean values of Achilles tendon hysteresis were small, suggesting that elastic property dominates in Achilles tendon viscoelastic properties, and that Achilles tendon efficiently stores and returns energy during cyclic loading. Large inter-individual variation was observed and negative values were also measured (implying energy release from tendon). These findings suggest that there may be methodological challenges in tendon hysteresis measurements in vivo. The possible sources of error are discussed in detail elsewhere (Finni et al. 2012), and here it is only mentioned that synchronization of force and ultrasound data or differential contributions of individual triceps surae or other plantarflexor muscles in the loading and unloading parts of the cycle may contribute to the possible error. If the errors associated with the measurement do not systematically bias the results in either direction, it can be concluded that Achilles tendon hysteresis in vivo in fast cyclic contractions is probably small. This would be in line with the functional role of the Achilles tendon, i.e. spring-like behavior involving storing and releasing energy, and also concurs with in vitro measurements (Ker 1981, Bennett et al. 1986).

6.3 Muscle architecture, tendon mechanical properties and physical function in old age

It was observed in the current thesis that plantarflexor muscle architecture and Achilles tendon stiffness were associated with performance in tests of physical function in older adults. These associations were found to be independent of plantarflexor and knee extensor muscle strength, lower extremity muscle mass and leg extension power. These findings are supported by earlier findings of a strong association between plantarflexor muscle function and walking performance in older adults (Suzuki et al. 2001, Bean et al. 2002), and also emphasize the functionally important role of muscle architecture and tendon stiffness.

In the multivariate models, shorter muscle fascicle length (MG for 6MWT and soleus for TUG) was independently associated with better performance in the tests of physical function. TT is responsible for the majority of length changes in triceps surae MTU during the stance phase of walking (Mian et al. 2007, Farris & Sawicki 2012, Lai et al. 2015). For this reason, long muscle fascicles may not provide a further advantage for force or power generation. Instead, individuals with short muscle fascicles may have better preserved triceps surae PCSA, since short fascicles allow for greater PCSA for a given muscle volume (Lieber & Friden 2001). Shorter fascicles may also reduce the energy cost of a given level of force generation due to lower activated muscle mass compared to a muscle with longer fascicles (Biewener & Roberts 2000). Finally, short fascicles may help to minimize the required muscle mass and thus overall energy requirements of swinging the lower leg during walking.

Better performance in 6MWT was found to be independently associated with greater Achilles tendon stiffness. It could be that walking faster requires a

stiffer Achilles tendon in order to maintain efficient function of triceps surae muscles (Lichtwark & Wilson 2007). Additionally, greater Achilles tendon stiffness in combination with greater triceps surae muscle strength may allow for greater elastic energy storage and utilization in walking, thereby improving performance in prolonged walking such as 6MWT.

6.4 Triceps surae muscle function during walking

When muscle-tendon function of young and older adults was compared at matched walking speeds, no significant differences were observed in any of the analyzed parameter related to triceps surae muscle function. However, when the groups were compared at their respective preferred speeds it was observed that older adults utilized narrower fascicle operating range, had lower fascicle shortening velocity at estimated peak force generation and that fascicle length changes accounted for lower proportion of MTU length changes compared to young adults. These results suggest that muscle mechanical function, such as velocity of shortening and muscle operating range, are dependent on walking speed without significant effect of age. In addition, these findings led to a hypothesis that the reason why older adults prefer slower walking speed compared to young adults is that it allows them to compensate for the loss of muscle strength in triceps surae muscles by improving muscle contractile conditions and enhancing use of TT elasticity. These factors may relate to minimizing metabolic cost of transport.

It was observed that at the preferred walking speed older adults had slower soleus and MG shortening velocity at estimated peak force generation compared to a faster walking speed matching the young adult's preferred speed. This allows a given force generation with lower muscle activation and hence energy consumption due to force-velocity relationship (Lichtwark & Wilson 2007). The lower activation required for a given force generation is supported by an observation that, in older adults, increase in walking speed by 22% was accompanied by 19% and 13% increases in MG and soleus RMS EMG during push-off, respectively, but only a 2% increase in peak resultant GRF. In addition, at preferred walking speed triceps surae muscles in older adults operated within a narrower fascicle operating range and TT length changes accounted for a greater proportion of the MTU length changes. The narrower fascicle operating range may contribute to the lower muscle activation for given force generation due to force-length relationship (Gordon et al. 1966). Moreover, narrower fascicle operating range and greater proportional use of TT elasticity suggest lower triceps suerae muscle work at the slower walking speed in older adults. These walking speed related adaptations in muscle function may provide a mechanism to mitigate the age-related reduction in plantarflexor force generation capacity and to reduce the energy cost of walking.

There was similar TT elongation during stance phase in walking between young and older adults but in older adults this was achieved with lower body weight normalized loading based on GRF measurements. This suggest that

there was a lower energy storage to TT (relative to body weight) in older adults that could lead to less effective catapult mechanism during push-off (Ishikawa et al. 2005) and hence to the lower peak ankle plantarflexion power observed previously (DeVita & Hortobagyi 2000). Older adults may compensate the lower elastic energy utilization by increasing power output from hip extensors in early stance (DeVita & Hortobagyi 2000) and by adopting a smoother center of mass transition over the stance leg with lower energy losses (Franz & Kram 2013).

Collectively the findings suggest that by choosing a slower walking speed older adults may optimize triceps surae muscle function from energetic perspective. Previous findings from normal weight and obese women suggest that minimizing cost of transport dictates selection of preferred walking speed (Browning & Kram 2005). This may be true for older adults too. Additionally, older adults may preferentially minimize energy cost of plantarflexors since this muscle group may be operating with the lowest reserve capacity among lower limb muscles in walking (van der Krogt et al. 2012, Beijersbergen et al. 2013).

Previous studies have shown differences between young and older adults walking at matched speed in soleus operating length (Panizzolo et al. 2013) and LG fascicle and TT contribution to MTU length change (Mian et al. 2007). The discrepancy between the results of the current thesis and previous studies may be due to the fact that in the current thesis spatio-temporal gait parameters were matched between the groups whereas they differed in previous studies. The similarity of spatio-temporal gait parameters between young and older adults at the matched walking speed can be considered as strength of the current study since it removes one confounding factor from the matched speed comparisons.

6.5 Limitations

The main limitation of the current thesis is cross-sectional study design utilized. The limitation of cross-sectional studies is inability to reveal cause and effect relationships and possible confounding of the results by factors other than the primary interest. Thus, the results provided by the current thesis cannot be interpreted to represent age-related changes in triceps surae muscle-tendon properties or function. To reveal the age-related changes, longitudinal studies should be conducted and to reveal effects of muscle-tendon properties on physical function, well designed randomized controlled should be conducted.

Another limitation of the current thesis is the limited number of subjects included. The limited sample size with high inter individual variability in some parameters resulted in modest statistical power. However, if significant age-related difference was unnoted due to low statistical power the functional relevance of such difference is probably small.

There are some limitations associated with muscle architectural measurements. Ideally, comparisons of muscle architectural parameters between groups should be made at similar sarcomere lengths. However, since it was not possi-

ble to obtain sarcomere lengths, muscle architectural parameters were measured at anatomical position (i.e. 90 degree ankle angle and knee extended). Interpretations of muscle function based on muscle architecture may be biased if there was systematic difference between groups in sarcomere lengths at which the measurements were taken e.g. due to differences in passive tension at anatomical position. Ultrasound imaging is sensitive to probe orientation and pressure between probe and skin (Klimstra et al. 2007, Bénard et al. 2009, Bolsterlee et al. 2016) but these factors are not likely to cause systematic errors that would bias only some group of subjects invalidating group comparisons. Finally, in the current thesis only single image was taken to reflect muscle architecture of a specific muscle and multiple images could improve validity of the measurement.

Finally, the older athletes in the current thesis included only men and also muscle-tendon function during walking was studied only from men. This may be significant limitation since sex-specific hormonal changes with aging may alter effects of aging and physical loading on muscle and tendon (Finni et al. 2009, Pöllänen et al. 2015).

7 PRIMARY FINDINGS AND CONCLUSIONS

In the current thesis only small to minor age-related differences in triceps surae muscle architecture (muscle fascicle length and pennation angle) were observed between healthy adults aged 18-30 and 70-80 years. More consistent differences were observed in measures of muscle size and especially muscle strength. In addition aging was observed to be associated with larger Achilles tendon cross-sectional area.

Findings from older athletes suggest that the observed age-related differences in triceps surae muscle architecture and Achilles tendon properties are probably not due to lack of physical loading but reflect primary aging or other age-related factors than reduced levels of physical activity. Exceptions may be muscle mass and muscle strength that could be partly reduced due to lack of high intensity loading to triceps surae muscles.

Relatively large inter individual variation in muscle architectural parameters and tendon mechanical properties raise the possibility that individuals differences in muscle architecture or tendon mechanical properties may have effect on muscle function and explain differences in physical function between older adults. The associations found between physical function and triceps surae muscle-tendon properties in older adults support this.

Finally, it was found that muscle-tendon function is highly conserved in older adult regardless of markedly reduced muscle strength. This highlight redundancy in neuromuscular system and suggest that the patterns of muscle-tendon interaction observed may provide optimal function of the muscle-tendon unit which is therefore beneficial to be conserved regardless of possible age-related changes in muscle-tendon properties and nervous system structure and control (Power et al. 2013b).

It is proposed, based on the results of the current thesis, that the age-related differences in triceps surae muscle architecture and in Achilles tendon properties could be seen as an adaptations of the muscle-tendon unit that compensate age-related loss of muscle mass and strength in order to maintain function of the muscle-tendon unit with aging.

In the future it should be investigated if targeted exercise interventions can influence structure and function of lower limb muscle-tendon unit and im-

prove muscle-tendon interaction to preserve or improve physical function in older adults. Evaluation of effectiveness of interventions designed to improve physical function in older adults should include biomechanical measurements including analysis of kinematics and kinetics, energetics, muscle-tendon function and musculoskeletal modeling to improve understanding of the mechanisms responsible for possible improvements or to explain why the intervention does not give the results hoped for.

Finally, although muscle-tendon mechanics are important for muscle function, and improving the interaction between muscle and tendon may be important for physical functioning in older adults, the results of the current thesis suggest that the impairments in muscle function are mainly due to loss of muscle mass and strength. These can be addressed with appropriate strength training. Due to the importance of plantarflexors for human locomotion strength training for plantarflexors is advised for older adults to maintain and improve physical function.

YHTEENVETO (FINNISH SUMMARY)

Pohjelihasten rakenne ja toiminta ikääntyessä

Ikääntyminen on yhteydessä lihasmassan katoon, joka johtaa lihastoiminnan heikentymiseen. Lihastoiminnan heikentyminen ilmenee esimerkiksi lihasvoiman ja tehontuoton heikentymisenä. Nämä muutokset saattavat johtaa liikuntakyvyn rajoituksiin sekä itsenäisen liikuntakyvyn menettämiseen.

Aikaisemmat tutkimukset ovat osoittaneet, että ikääntymiseen liittyvä lihastoiminnan heikkeneminen ei johdu pelkästään lihasmassan kadosta. Osoituksena tästä on lihasvoiman ja tehontuoton nopeampi heikkeneminen suhteessa lihasmassan vähenemiseen. Osasyynä lihastoiminnan suurempaan heikkenemiseen verrattuna lihasmassan katoon saattaa olla muutokset lihaksen rakenteellisissa ominaisuuksissa tai lihaksen luustoon kiinnittävässä jänteessä. Lihaksen rakenteellisia ominaisuuksia kutsutaan lihasarkkitehtuuriksi. Se määrittelee, miten lihassolut lihaksen sisällä ovat järjestäytyneet. Lihasarkkitehtuurilla ja jänteen mekaanisilla ominaisuuksilla, kuten jäykkyydellä, on merkittävä rooli lihaksen toiminnan kannalta. Jänteen ominaisuuksien rooli korostuu erityisesti pohjelihaksissa, jotka kiinnittyvät pitkään ja elastiseen akillesjänteeseen.

Lihaksen arkkitehtuurissa ja jänteen mekaanisissa ominaisuuksissa on aikaisemmissa tutkimuksissa havaittu eroja eri ikäryhmien välillä. Näiden havaintojen perusteella on mahdollista, että lihaksen arkkitehtuurissa tai jänteen mekaanisissa ominaisuuksissa tapahtuvilla muutoksilla on rooli ikääntymiseen liittyvässä lihastoiminnan heikkenemisessä ja liikkumiskyvyn laskussa. Aikaisemmat tutkimustulokset liittyen lihasarkkitehtuurin tai jänteen ominaisuuksien ikääntymismuutoksiin ovat kuitenkin osittain ristiriitaisia, eikä tiedetä, miten ikääntyminen vaikuttaa pohjelihasten lihasarkkitehtuuriin tai akillesjänteen ominaisuuksiin. Lisäksi näiden mahdollisten ikääntymiseen liittyvien muutosten toiminnallisesta roolista tiedetään toistaiseksi vähän.

Tämän väitöskirjan tarkoituksena oli selvittää ikääntymiseen liittyviä muutoksia lihasten arkkitehtuurissa ja jänteen ominaisuuksissa sekä selvittää näiden lihaksen toimintaan vaikuttavien tekijöiden merkitystä ikääntyneiden liikkumiskyvyn kannalta. Tutkimuskohteeksi valittiin pohjelihakset niiden liikkumisen kannalta tärkeän roolin takia.

Tähän väitöskirjaan kuuluviin osatutkimuksiin osallistui yhteensä 143 tervettä 18-30- tai 70-80-vuotiasta henkilöä. Osa tutkittavista oli yleisurheilun maailmanmestaruuskilpailuihin osallistuneita veteraaniurheilijoita. Tutkimuksen tulokset osoittivat, että 70-80-vuotiailla henkilöillä oli selkeästi alhaisempi pohjelihasten maksimaalinen voimantuottokyky verrattuna 18-30-vuotiaisiin henkilöihin. Kyseisellä ikääntyneiden ryhmällä pohjelihakset olivat myös kooltaan pienemmät verrattuna nuorten ryhmään. Pohjelihasten arkkitehtuurissa havaittiin vain pieniä eroja, joista merkittävin oli 8 % lyhyempi kaksoiskantalihaksen lihassolukimppujen pituus. Akillesjänteen ominaisuuksia verratessa havaittiin ikääntyneillä olevan poikkipinta-alaltaan suuremmat jänteet, mutta jänteen jäykkyys oli alhaisempi. Näin ollen ikääntyneillä jänne oli materiaalina mekaanisilta ominaisuuksiltaan erilaista (alhaisempi moduuli) verrattuna nuoriin.

Kun vastaavia pohjelihaksen arkkitehtuurin ja akillesjänteen ominaisuuksia verrattiin 70-80-vuotiaiden veteraaniurheilijoiden ja nuorten sekä ikääntyneiden terveiden, mutta harjoittelemattomien henkilöiden välillä havaittiin vain muutamia eroavaisuuksia ryhmien välillä. Kestävyysjuoksua harrastavilla ikääntyneillä oli lyhyemmät leveän kantalihaksen lihassolukimput verrattuna nuoriin ja ikääntyneisiin harjoittelemattomiin henkilöihin. Pikajuoksua harrastavilla ikääntyneillä henkilöillä oli puolestaan suurempi akillesjänteen poikkipinta-ala verrattuna molempiin harjoittelemattomiin ikäryhmiin.

Ikääntyneiden liikkumiskyvyn havaittiin olevan yhteydessä pohjelihasten arkkitehtuuriin sekä akillesjänteen jäykkyyteen. Suurempi akillesjänteen jäykkyys ja lyhyemmät kaksoiskantalihaksen lihassolukimput olivat yhteydessä kuuden minuutin kävelytestin aikana kuljettuun matkaan riippumatta alaraajojen lihasmassasta, pohjelihasten voimantuottokyvystä tai alaraajojen ojentajalihasten tehontuottokyvystä. Vastaavasti lyhyemmän ajan ”timed up-and-go” –testissä havaittiin olevan yhteydessä lyhyempiin leveän kantalihaksen lihassolukimppuihin.

Tarkastellessa lihaksen ja jänteen yhteistoimintaa pohjelihaksissa, ei ikääntyneiden ja nuorten välillä havaittu eroja verrattaessa ryhmiä samalla kävelynopeudella. Kun ryhmiä verrattiin molempien omalla luonnollisella kävelynopeudella, joka oli ikääntyneillä alhaisempi, havaittiin, että ikääntyneillä pohjelihasten lihassolukimppujen supistumisnopeus arvioidun maksimaalaisen voimantuoton aikana oli alhaisempi verrattuna nuoriin ja lisäksi jänteisten kudosten elastisuuden hyödyntäminen oli suurempaa.

Tämän väitöskirjan tulokset antavat uutta tietoa ikääntymiseen liittyvän lihastoiminnan heikentymisen syistä. Pohjelihasten arkkitehtuurissa tai akillesjänteen ominaisuuksissa tapahtuvat muutokset eivät todennäköisesti ole merkittävässä roolissa lihastoiminnan suuremmassa heikkenemisessä suhteessa lihasmassan vähenemiseen. Päinvastoin, lihaksen arkkitehtuurin ja jänteen mekaanisten ominaisuuksien mahdolliset ikääntymismuutokset saattavat olla osa adaptaatioita, jotka pyrkivät ylläpitämään lihaksen toiminnallisuuden lihasmassan vähentyessä. Koska jänteen ja lihaksen yhteistoiminta näyttää säilyvän kävelyn aikana vastaavanlaisena nuoriin verrattuna, pohjelihasten toiminnan heikentyminen kävelyssä ei näytä johtuvan lihaksen ja jänteen yhteistoiminnan muutoksista. Pohjelihasten lihasvoiman heikkeneminen saattaa kuitenkin johtaa siihen, että akillesjänteeseen varastoituva energia askeleen aikana jää ikääntyneillä pienemmäksi, joka mahdollisesti vaikuttaa negatiivisesti nilkan tehontuottoon askeleen työntövaiheessa. Kävelemällä hitaammin ikääntyneet mahdollisesti kompensoivat pohjelihasten heikentynyttä voimantuottokykyä. Tästä syystä pohjelihasten voimaharjoittelu on suositeltavaa ikääntymiseen liittyvän liikuntakyvyn heikkenemisen ehkäisyssä.

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ORIGINAL PAPERS

I

AGE-RELATED DIFFERENCES IN ACHILLES TENDON PROPERTIES AND TRICEPS SURAE MUSCLE ARCHITECTURE IN VIVO

by

Stenroth L, Peltonen J, Cronin NJ, Sipilä S & Finni T, 2012

Journal of Applied Physiology 113(10), 1537-1544

J Appl Physiol 113: 1537–1544, 2012.
First published October 4, 2012; doi:10.1152/japplphysiol.00782.2012.

Age-related differences in Achilles tendon properties and triceps surae muscle architecture in vivo

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Submitted 25 June 2012; accepted in final form 28 September 2012

Stenroth L, Peltonen J, Cronin NJ, Sipilä S, Finni T. Age-related differences in Achilles tendon properties and triceps surae muscle architecture in vivo. *J Appl Physiol* 113: 1537–1544, 2012. First published October 4, 2012; doi:10.1152/japplphysiol.00782.2012.—This study examined the concurrent age-related differences in muscle and tendon structure and properties. Achilles tendon morphology and mechanical properties and triceps surae muscle architecture were measured from 100 subjects [33 young (24 ± 2 yr) and 67 old (75 ± 3 yr)]. Motion analysis-assisted ultrasonography was used to determine tendon stiffness, Young's modulus, and hysteresis during isometric ramp contractions. Ultrasonography was used to measure muscle architectural features and size and tendon cross-sectional area. Older participants had 17% lower ($P < 0.01$) Achilles tendon stiffness and 32% lower ($P < 0.001$) Young's modulus than young participants. Tendon cross-sectional area was also 16% larger ($P < 0.001$) in older participants. Triceps surae muscle size was smaller ($P < 0.05$) and gastrocnemius medialis muscle fascicle length shorter ($P < 0.05$) in old compared with young. Maximal plantarflexion force was associated with tendon stiffness and Young's modulus ($r = 0.580$, $P < 0.001$ and $r = 0.561$, $P < 0.001$, respectively). Comparison between old and young subjects with similar strengths did not reveal a difference in tendon stiffness. The results suggest that regardless of age, Achilles tendon mechanical properties adapt to match the level of muscle performance. Old people may compensate for lower tendon material properties by increasing tendon cross-sectional area. Lower tendon stiffness in older subjects might be beneficial for movement economy in low-intensity locomotion and thus optimized for their daily activities.

aging; muscle structure; tendon biomechanics

IN OLDER PEOPLE, MUSCLE STRENGTH and power are important determinants of independent living and are associated with functional status and fall incidence (18, 53). Aging is associated with a marked loss in muscle force (41, 49) and power (50), as well as changes in tendon properties (44) and muscle architecture (38). Tendinous tissue properties and muscle architecture have a marked effect on muscle function (42), but the role of these changes in lowered muscle function with aging is not clear as there are inconsistent findings in the area of aging and muscle-tendon complex properties.

Research examining the association between aging and tendon properties is inconclusive. Studies have shown similar (23, 25) or lower (39, 44) Achilles tendon stiffness and similar (9, 11) or lower (23, 25) patella tendon stiffness in old compared with young subjects. No consistent differences in tendon cross-sectional area (CSA) have been found (9, 11, 36, 44). Findings of age-related differences in muscle architecture are also inconclusive as others report differences in pennation angle and fascicle length in triceps surae (38, 43) but others fail to observe such differences (23, 24). Discrepancy between the studies in the association between aging and muscle-tendon complex properties could be related to small sample size or methodological differences. We aim to avoid these problems with a representative sample of healthy young and old subjects and using state-of-the-art methods.

A study examining age-related differences in muscle and tendon is the first step toward understanding the role of mechanical properties of muscle-tendon complex on age-related decline in muscle performance. It is important to study both muscle and tendon in the same study to prevent sampling-related bias when trying to conclude how aging affects muscle-tendon complex properties.

This study was set to examine differences in Achilles tendon properties and triceps surae muscle architecture between healthy old and young individuals. Both men and women were studied because of possible sex differences in tendon properties (45), and a substantially larger sample size was used compared with previous studies to account for individual variation. Therefore, this study will significantly increase the knowledge on aging muscle-tendon complex. We hypothesized that Achilles tendon stiffness and Young's modulus would be lower, Achilles tendon CSA similar, triceps surae muscle size and pennation angle lower, and muscle fascicle length lower in gastrocnemius medialis but not in soleus in old compared with young individuals. We further hypothesize that men and women do not differ in muscle architecture, but women have lower tendon mechanical properties and smaller muscle size and tendon CSA.

METHODS

Subjects. Thirty-three 18- to 30-yr-old young (18 men and 15 women) and 67 70- to 80-yr-old elderly (33 men and 34 women) subjects were recruited to the study (Table 1). Young subjects were university students. Older subjects were recruited from the University of Third Age or from weekly meetings of retired people.

Using telephone interviews an equal number of healthy sedentary and active older subjects were recruited to obtain a representative sample of aged people with varying physical activity levels. Sedentary was defined as a person exercising for fitness and health one or less times per week. Active was defined as a person who exercised three or more times per week (30 min or more with intensity sufficient to cause sweating or breathlessness).

Subjects did not train for competitive sports or participate in other scientific studies at the time of testing. Subject exclusion criteria were Achilles tendon pain, history of Achilles tendon rupture or surgery, neurologic and progressive severe illnesses, insulin-treated diabetes, fracture within the previous year, immobilization for 1 wk during the last 3 mo, daily use of painkillers, use of immunosuppressive drugs or anticoagulants, medical treatment for cancer within the last year, severe visual or hearing impairment and mini mental state examina-

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Table 1. *Subject characteristics*

	Age, yr	Height, cm	Body weight, kg	BMI, kg/m^2	Plantarflexion MVC, N
Young (n = 33)	24.1 ± 2.4	174 ± 9**	69.5 ± 10.0	23.0 ± 2.3**	1,398 ± 356**
Old (n = 67)	74.6 ± 3.4	166 ± 9	69.8 ± 10.2	25.2 ± 2.6	859 ± 291
Men (n = 51)	56.8 ± 24.9	176 ± 6††	75.9 ± 8.1††	24.6 ± 2.7	1,210 ± 379††
Women (n = 49)	59.1 ± 23.4	161 ± 6	63.3 ± 7.8	24.4 ± 2.8	857 ± 347
YM (n = 18)	23.7 ± 2.0	181 ± 6	75.4 ± 9.0	23.1 ± 2.6	1,541 ± 376
OM (n = 33)	74.8 ± 3.6	173 ± 5	76.1 ± 7.7	25.4 ± 2.4	1,029 ± 232
YW (n = 15)	24.5 ± 2.8	166 ± 4	62.5 ± 5.6	22.8 ± 1.8	1,226 ± 246
OW (n = 34)	74.3 ± 3.3	159 ± 5	63.7 ± 8.6	25.1 ± 2.9	694 ± 246

Values are expressed as means ± SD. YM, young men; OM, old men; YW, young women; OW, old women; BMI, body mass index; MVC, maximal voluntary contraction. Asterisks are for young vs. old; daggers are for men vs. women: **, ††$P < 0.01$.

tion score of 23 or lower. Self-reported health status and medication were confirmed by a physician for the older subjects and a research nurse for the younger participants during a clinical examination.

Participants were informed about the procedures used in the study and they all signed a written consent prior to the study. The local ethical committee approved all methods and the study conformed to the standards set by the latest revision of the Declaration of Helsinki.

Measurements. Achilles tendon CSA and length, triceps surae muscle architecture and gastrocnemius CSA were first measured with ultrasonography (US; Aloka Pro Sound alpha 10). Achilles tendon mechanical properties where then measured in an ankle dynamometer during plantarflexion contractions using ultrasonography combined with motion analysis. All measurements were taken from the right leg by the same experienced researcher.

Achilles tendon cross-sectional area and length. Subjects lay prone on a table with the ankle kept at 90° by hand while Achilles tendon CSA was measured with a 3.6-cm linear probe (UST-5411, Aloka, Japan) and an acoustic gel pad (SonarAid, Geistlich Pharma). Ultrasound image of Achilles tendon CSA was taken at rest from transverse plane, four centimeters proximal from the proximal margin of calcaneal tubercle (Achilles tendon insertion site) which is approximately the narrowest site of free Achilles tendon (48). CSA was outlined using a polygon selection tool (Fig. 1). The most distal point of gastrocnemius medialis (GM) muscle-tendon junction (MTJ) and the distal insertion of Achilles tendon were visualized using ultrasonography and marked on to the skin. Achilles tendon resting length was measured as the distance between these two points using a ruler.

Muscle architecture. Muscle architecture was measured from GM and soleus (SOL) and the measurements were performed within 10 min of the subject lying down to prevent fluid redistribution affecting the results (10). Imaging was done at 50% of GM muscle length using a 6-cm linear probe (UST-5712, Aloka, Japan). Imaging locations were optimized for fascicle imaging and were mid muscle in the medial-lateral direction for both GM and SOL (22). For both muscles an acoustic gel was used between the skin and the probe, and the probe was held gently over the skin without applying pressure to the tissues underneath. From the images GM and SOL muscle thickness, fascicle length, and pennation angle were measured. Muscle thickness was measured by drawing a perpendicular line from the deep to superficial aponeurosis at the center of the image (Fig. 1). Muscle fascicle length was measured by drawing a line along a clearly visible muscle fascicle between deep and superficial aponeurosis. If the fascicle was not apparently straight, the curvature of the fascicle was taken into account by drawing the line with multiple points. If necessary, fascicle length was measured by extrapolating the excursion of the fascicle from superficial to deep aponeurosis (15). Fascicle pennation angle was measured as the angle between muscle fascicle and deep aponeurosis. Normalized fascicle length in the direction of muscle pull (referred to later as normalized fascicle length) was calculated by multiplying fascicle length with cosine of the pennation angle divided by the length of the tibia measured with a ruler. This allowed us to compare the functional fascicle length between the study groups.

Muscle anatomical CSA. Gastrocnemius (lateral and medial head) anatomical CSA was measured at 50% of GM length using panoramic US-scan with a 3.6-cm linear probe (UST-5411, Aloka, Japan). This method has been described and validated for quadriceps muscles, and is reproducible and suitable for group comparisons (2). A sequence of images was taken in extended field-of-view mode and the ultrasound device combined these images to one panoramic view of the gastrocnemius muscles. Soleus muscle was not included in the muscle cross-sectional measurement because the borders of that muscle are hard to identify from panoramic images, especially from older subjects. For the CSA analyses a polygon selection tool was used to outline the muscles manually (Fig. 2).

Image analysis for tendon morphology, muscle architecture, and muscle CSA were made using an open source computer program (ImageJ 1.44b, National Institutes of Health). Analyses were done twice by the same investigator on separate days, and a mean was used for further analysis (intraclass correlation ranged from 0.910 to 0.996 and typical error ranged from 0.9 to 6.5%).

Tendon mechanical properties. Plantarflexions were performed in a custom-made dynamometer (University of Jyväskylä) operated in a fixed position (48). Subjects were seated in the dynamometer with the right ankle at 90° of flexion, knee fully extended and hip at 60° of

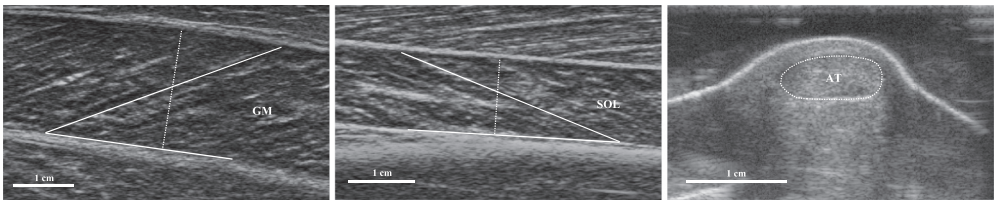

Fig. 1. From *left* to *right*: gastrocnemius medialis (GM) muscle architecture, soleus (SOL) muscle architecture and Achilles tendon (AT) cross-sectional area (outlined). Fascicle length (solid line along fascicles), pennation angle (angle between solid lines) and muscle thickness (dotted line) are drawn in the images of GM and SOL. The images shown are from a young woman.

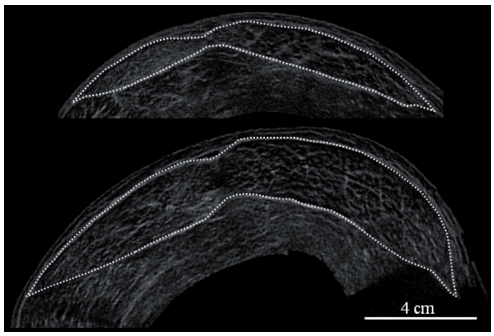

Fig. 2. Gastrocnemius muscle cross-sectional area outlined from an old (*top*) and a young (*bottom*) male subject.

flexion (full extension 0°). The seat was set individually as close to the pedal as possible in knee extended position, which minimized ankle joint rotation during maximal plantarflexion as the subject was tightly fixed between the pedal and the back rest. Velcro straps held knee extended and foot attached to the pedal. A monitor was placed in front of the subjects where they could follow the force signal in real time.

In practice trials subjects performed a series of plantarflexions by pushing the pedal with the ball of their foot following verbal commands. Each contraction in the series lasted for 1 s with rise and fall of the force occurring at the same rate. These contractions have been shown to provide better reproducibility of force production than slower contractions without affecting stiffness or hysteresis (47). Two to three sets of five to eight contractions were performed with an instruction to use half of their maximal force. Maximal voluntary contraction (MVC) force of the plantarflexors was measured three times (3-s contractions) with strong verbal encouragement. The maximal reaction force of the pedal obtained in MVC measurements is hereafter referred to as MVC force.

The best MVC was used to calculate 80% of MVC force. Practice trials and MVC measurements were considered to stabilize Achilles tendon mechanical properties before the actual measurement (32). Tendon properties were calculated from a set of five to eight plantarflexions to a peak force level of at least 80% MVC (Fig. 3). A horizontal line on the screen at the 80% MVC level served as a visual guide.

To derive the force-elongation relationship of the Achilles tendon, plantarflexion force, heel displacement, MG MTJ displacement and movement of the ultrasound probe were simultaneously measured and synchronized with a TTL pulse as described later. The tendon stress-strain relationship was calculated using the tendon force-elongation relationship and anthropometric data.

Plantarflexion force was measured with a force transducer (Precision TB5-C1, Raute, Nastola, Finland) installed to the pedal of the dynamometer. Force signals were sampled with a 16-bit AD-board (Power 1401, CED) at 1 kHz. Achilles tendon force was calculated by multiplying the measured reaction force with the ratio between the externally measured lever arms of the foot and the Achilles tendon. For measuring foot lever arm, subjects placed their right foot on to a paper that had a scale printed on it. The foot longitudinal axis was perpendicular to the scale. The vertical projections of the outermost tip of the medial malleolus and the head of the first metatarsal were marked on to the paper and the distance between these point was determined as the lever arm of the foot. Achilles tendon lever arm was defined as the distance from the center of the Achilles tendon to the outermost tip of the medial malleolus in sagittal plane measured using a ruler.

Displacement of the both ends of the Achilles tendon during the trials was measured to determine Achilles tendon length. The displacement of the distal end of the Achilles tendon in the sagittal plane was measured using a potentiometer installed under the heel with a sampling frequency of 1 kHz. The potentiometer measures the linear distance of the heel from the pedal and is able to detect heel displacement of under 0.1 mm. The displacement of the proximal end of the Achilles tendon was recorded with US at a sampling frequency of 70 Hz using a 6-cm linear array probe (UST-5712, Aloka, Japan). The probe was positioned over the GM MTJ, 2 cm medial from the border between medial and lateral gastrocnemius and secured with an elastic band. An acoustic gel pad (SonarAid, Geistlich Pharma) was used between the probe and the skin. Automatic tracking software was used to analyze MTJ displacement in the US image. The software is based on a pyramidal implementation of the Lukas-Kanade feature tracking (8). Nine tracking points were placed along the aponeurosis between GM and SOL, just proximal to the GM MTJ. The tracking algorithm has been previously shown to have a repeatability of 98% (35). Trials were analyzed twice and the mean was used for further analyses.

2D motion analysis was performed to measure the small movement of the ultrasound probe in the sagittal plane. Four reflective markers were placed on the handle of the probe to enable tracking of linear and rotational movements. A single high-speed video camera (InLine 250, Fastec Imaging) recorded movement perpendicular to the axis of motion at 60 Hz. The reflective markers were digitized from the video files using Peak Motus 2000 software (Peak Performance Technologies) and their location in relation to the laboratory coordinate system was determined using a rigid calibration object.

Tendon length during the trials was quantified by combining GM MTJ displacement in ultrasound frame, ultrasound probe movement, and heel displacement. GM MTJ location at the ultrasound image coordinate system was transformed to laboratory coordinate system using the location and orientation information of the ultrasound probe obtained from motion analysis. The location of the Achilles tendon insertion to the calcaneus in laboratory coordinate system was determined using the data from potentiometer and the measured distance from under the foot to the proximal margin of calcaneal tubercle. As both ends of the MG, tendon was determined in the same coordinate system; it was possible to determine the tendon length as the distance between those two points and elongation by subtracting tendon length at rest. Data synchronization and calculations were made in Matlab software (version R2010b9, The MathWorks) using custom-made

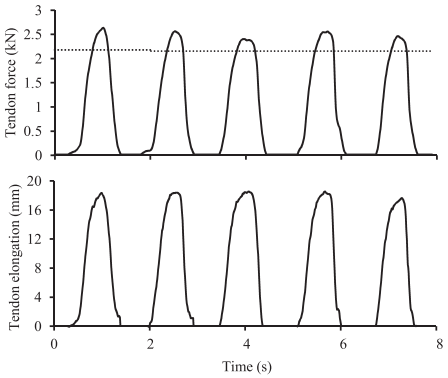

Fig. 3. An example of a trial from which tendon properties were calculated. Tendon force at the top and tendon elongation at the bottom. Horizontal line marks the 80% maximal voluntary contraction (MVC) force level.

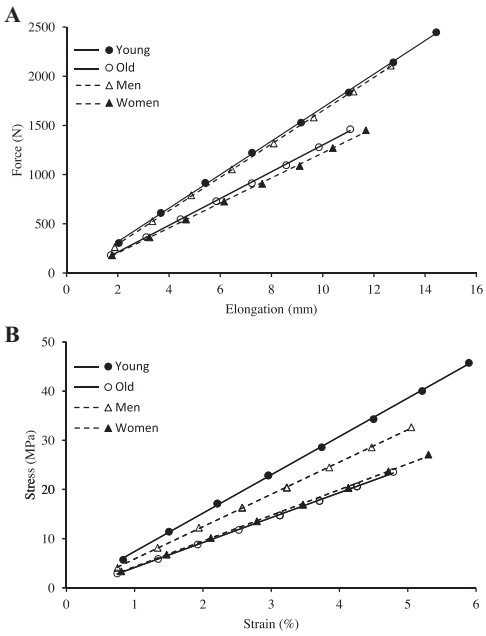

Fig. 4. Mean Achilles tendon force-elongation (*A*) and stress-strain (*B*) plots for young ($n = 33$), old ($n = 67$), men ($n = 51$), and women ($n = 49$). Lines are linear fits and represent Achilles tendon stiffness and Young's modulus, respectively. Values are calculated at 10% MVC increments from 10 to 80% MVC.

scripts. For each of the contractions, tendon force and elongation data were time normalized and averaged to produce one force-elongation relationship for every subject.

Tendon stiffness and Young's modulus were calculated between 10% and 80% of MVC force. This range was chosen since it was not possible for the subjects to produce MVC force with repeated contractions. Within this region, force-elongation curves were linear (Fig. 4*A*; $r = 0.999$). Stiffness was calculated as the slope of the force-elongation relationship and Young's modulus as the slope of the stress-strain relationship using the least-squares fit method. Stress was calculated by dividing tendon force by free tendon CSA (measurement described earlier), and strain was calculated by normalizing tendon elongation to tendon length at rest. Tendon hysteresis was calculated by using the full force-elongation curves and was defined as the percentage difference in the area under the curve for the loading and unloading phases.

Statistical analyses. Differences between the study groups (old vs. young and men vs. women) were tested using two-way ANOVA. Age, sex, and the age × sex interaction were considered independent variables. Homogeneity of variances was tested using Levene's test and normality of distributions was tested using Kolmogorov-Smirnov test. If homogeneity or normality assumptions were violated, the results of the variance analysis were confirmed using nonparametric tests. Only the results from variance analysis are reported since the results from nonparametric tests were in accordance with the variance analysis. For comparing tendon elongation and strain between the groups in similar absolute force values we chose to use a value that has real life relevance and used the mean peak Achilles tendon force value reported for walking (16) that is 1.32 kN. For comparison of tendon stiffness and Young's modulus between young and old subjects with similar muscle strengths we used subjects that had MVC force at a range mean ± SD of MVC forces of the whole population. Student's two-tailed independent samples *t*-test was used for this comparison. The degree of association between variables was tested using Pearson's product-moment correlation. The level of statistical significance was set at $\alpha = 0.05$ for all tests.

RESULTS

Significant interactions between age and sex were not found for any variables. The individual group means are presented in the Tables 1, 2, and 3.

Muscle architectural features and size. SOL pennation angle was larger in men compared with women (18%, $P < 0.001$). Fascicle length in GM was shorter in old compared with young (7%, $P < 0.05$). Muscle thickness was significantly lower in old compared with young in SOL and in GM (9%, $P < 0.05$ and 13% and $P < 0.001$, respectively). Gastrocnemius muscle CSA was 15% smaller in old compared with young subjects ($P < 0.01$) and 12% smaller in women compared with men ($P < 0.01$, Table 3). GM and SOL normalized fascicle length was significantly different between men and women (GM 0.108 ± 0.020 vs. 0.118 ± 0.021, $P < 0.05$; SOL 0.094 ± 0.020 vs. 0.116 ± 0.030, $P < 0.01$), but there was no difference between young and old (GM 0.115 ± 0.020 vs. 0.112 ± 0.022, $P = 0.327$; SOL 0.103 ± 0.028 vs. 0.106 ± 0.027, $P = 0.548$).

Tendon properties. Achilles tendon CSA was 16% larger in old compared with young subjects ($P < 0.001$) and 21% larger in men compared with women ($P < 0.001$, Table 2). Achilles tendon stiffness was lower in old compared with young subjects (17%, $P < 0.01$) and women compared with men (25%, $P < 0.001$). Young's modulus was lower in old compared with young subjects (32%, $P < 0.001$) and women compared with men (19%, $P < 0.01$ Fig. 4, Table 3). There was a significant

Table 2. *Achilles tendon properties*

	Resting length, cm	CSA, mm^2	Stiffness, N/mm	Young's Modulus, GPa	Hysteresis, %
Young ($n = 33$)	18.7 ± 2.6*	53.49 ± 9.75**	170 ± 37**	0.79 ± 0.20**	3.0 ± 5.2
Old ($n = 67$)	17.4 ± 2.6	61.98 ± 12.64	141 ± 48	0.54 ± 0.18	2.5 ± 5.1
Men ($n = 51$)	19.2 ± 2.4††	64.58 ± 12.79††	171 ± 45††	0.68 ± 0.22††	2.3 ± 4.2
Women ($n = 49$)	16.3 ± 2.0	53.56 ± 9.09	129 ± 38	0.55 ± 0.21	3.1 ± 5.9
YM ($n = 18$)	19.7 ± 2.6	56.53 ± 9.64	186 ± 37	0.86 ± 0.20	1.4 ± 3.7
OM ($n = 33$)	19.0 ± 2.2	68.97 ± 12.24	164 ± 47	0.59 ± 0.17	2.8 ± 4.4
YW ($n = 15$)	17.4 ± 1.9	49.84 ± 8.83	151 ± 29	0.71 ± 0.18	5.0 ± 6.0
OW ($n = 34$)	15.9 ± 1.9	55.20 ± 8.83	120 ± 39	0.48 ± 0.18	2.3 ± 5.7

Values are expressed as means ± SD. CSA, cross-sectional area. Asterisks are for young vs. old; daggers are for men vs. women: *$P < 0.05$; **, ††$P < 0.01$.

Table 3. *Muscle architectural features and gastrocnemius cross-sectional area*

	SOL angle, °	SOL fl, mm	SOL thickness, mm	GM angle, °	GM fl, mm	GM thickness, mm	G CSA, cm^2
Young (n = 33)	20.24 ± 5.47	41.10 ± 9.91	13.85 ± 2.63*	24.90 ± 3.95	47.51 ± 6.68*	19.73 ± 2.64*	22.56 ± 4.69**
Old (n = 59/67)	18.77 ± 4.36	39.84 ± 7.90	12.54 ± 2.99	23.78 ± 3.96	43.96 ± 7.17	17.18 ± 2.72	19.13 ± 4.42
Men (n = 48/51)	21.12 ± 4.63††	39.36 ± 8.01	13.56 ± 2.66†	24.57 ± 4.13	45.97 ± 7.33	18.59 ± 3.18†	21.54 ± 4.90††
Women (n = 44/49)	17.32 ± 4.20	41.22 ± 9.23	12.40 ± 3.12	23.65 ± 3.80	44.09 ± 6.89	17.37 ± 2.52	18.89 ± 4.27
YM (n = 18)	20.92 ± 5.82	40.67 ± 9.04	14.26 ± 2.63	25.04 ± 4.05	47.70 ± 6.84	20.17 ± 2.54	24.35 ± 4.61
OM (n = 30/33)	21.17 ± 3.94	38.72 ± 7.47	13.15 ± 2.62	24.40 ± 4.18	45.25 ± 7.61	17.81 ± 3.22	20.12 ± 4.46
YW (n = 15)	19.46 ± 5.12	41.58 ± 11.12	13.38 ± 2.64	24.74 ± 3.96	47.30 ± 6.74	19.23 ± 2.74	20.54 ± 4.01
OW (n = 29/34)	16.21 ± 3.21	41.04 ± 8.30	11.88 ± 3.26	23.17 ± 3.68	42.67 ± 6.56	16.55 ± 1.95	18.16 ± 4.23

Values are expressed as means ± SD. Number of subjects (n), SOL/GM; SOL/GM angle, soleus/gastrocnemius medialis pennation angle; fl, fascicle length; G CSA, gastrocnemius muscle cross-sectional area. Asterisks are for young vs. old; daggers (†) are for men vs. women; *, †, ‡$P < 0.05$; **, ††$P < 0.01$.

difference in elongation and strain at 80% MVC force between old and young subjects (old vs. young: 11.1 ± 3.4 vs. 14.4 ± 3.1 mm, $P < 0.001$ and 4.8 ± 1.5 vs. 6.0 ± 1.5%, $P < 0.001$), but not between men and women (men vs. women: 12.7 ± 3.3 vs. 11.7 ± 3.9 mm, $P = 0.451$ and 5.1 ± 1.4 vs. 5.3 ± 1.8%, $P = 0.191$). We also compared elongation and strain at tendon force of 1.32 kN corresponding to the peak tendon force in walking (16) and found that there was a significant difference between old and young and between men and women in both elongation and strain (old vs. young: 9.6 ± 2.4 vs. 8.1 ± 2.4 mm, $P < 0.01$ and 4.1 ± 1.0 vs. 3.4 ± 1.3%, $P < 0.01$; men vs. women: 8.4 ± 2.4 vs. 9.9 ± 2.3 mm, $P < 0.01$ and 3.4 ± 1.0 vs. 4.5 ± 1.1%, $P < 0.001$). Stress at MVC force was lower in old compared with young (29.6 ± 9.5 vs. 57.9 ± 14.7 MPa, $P < 0.001$) and women compared with men (35.2 ± 17.5 vs. 42.6 ± 17.0, $P = 0.05$). When comparing old and young subjects with similar MVC forces (at a range mean ± SD, old n = 50 and young n = 19), we observed similar stiffness (old vs. young: 153 ± 42.9 vs. 151 ± 29.0 N/mm, $P = 0.861$) but significantly different Young's modulus (old vs. young: 0.58 ± 0.17 vs. 0.73 ± 0.17 GPa, $P = 0.001$, Fig. 5).

Maximal isometric force. MVC force was 39% lower in old compared with young subjects (859 ± 291 vs. 1,398 ± 356 N, $P < 0.001$) and 29% lower in women compared with men (857 ± 347 vs. 1,210 ± 379 N, $P < 0.001$).

Associations between variables. The correlation coefficient between tendon stiffness and MVC force and between Young's modulus and MVC force was significant in combined data (r = 0.580, $P < 0.001$ and $r = 0.561$, $P < 0.001$, respectively; Fig. 5). In old subjects there was a significant correlation between stiffness and MVC ($r = 0.549$, $P < 0.001$) and between Young's modulus and MVC ($r = 0.376$, $P = 0.01$). In young subjects a significant correlation was found between stiffness and MVC (r = 0.535, $P = 0.001$). Tendon stiffness correlated significantly with gastrocnemius muscle CSA and body weight ($r = 0.219$, $P < 0.05$ and $r = 0.278$, $P < 0.01$, respectively), but Young's modulus was not correlated with either muscle CSA or body weight. Neither MVC force nor gastrocnemius muscle CSA was associated with tendon CSA.

DISCUSSION

This study provides novel information about changes in the muscle-tendon complex due to aging with the largest number of subjects to date measured with state-of-the-art methods of determining tendon properties. There were four main findings from this study: *1*) Achilles tendon stiffness and Young's modulus were lower in healthy old men and women compared with young subjects; *2*) Achilles tendon CSA was larger in old compared with young subjects despite lower tendon stiffness, maximal plantarflexion force, and gastrocnemius muscle CSA; *3*) Achilles tendon mechanical properties correlated with muscle force-producing capacity even in old age; and *4*) Achilles tendon stiffness did not differ between young and old individuals with similar strengths.

Achilles tendon CSA. To our knowledge the current study is the only one to show larger Achilles tendon CSA in old men and women compared with young. Our finding is supported by a previously study by Magnusson et al. (34) that showed larger Achilles tendon CSA in old compared with young women using MRI. The observed difference in tendon CSA in the current study cannot be attributed to body weight or tendon length, since body weight was similar between old and young and tendon length was smaller in old compared with young. Habitual exercise-related tendon hypertrophy (27, 51) also

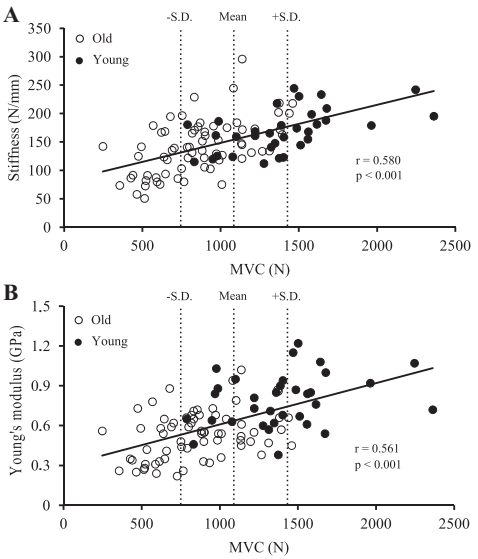

Fig. 5. Correlations between MVC force and stiffness (*A*) and MVC force and Young's modulus (*B*). Mean ± SD range of MVC force is marked with dotted lines. Within that range tendon stiffness did not differ, but Young's modulus was significantly different between young and old subjects.

seems an unlikely explanation because aging is associated with a decrease in moderate to vigorous physical activity (6) and the magnitude of tendon loading seems to be a key factor related to adaptive responses (4). One possibility is that a decrease in the loading of the tendon could increase extracellular water content and thus increase tendon CSA in old subjects (26). Another possible explanation for larger tendon CSA in old subjects is that pathological processes related to high levels of circulating cholesterol (7) could cause a large decrease in tendon material properties that has to be compensated by increased tendon CSA to maintain appropriate tendon stiffness. Some of the old subjects used statin medication for high levels of cholesterol, but we did not observe a difference in tendon CSA between statin users and nonusers (users: 19 old men and 13 old women). In women, estradiol concentration can affect tendon dimensions (17) and prevention of decrease in estradiol concentration by hormone replacement therapy (HRT) might hinder the growth of tendon CSA in older women. In the current study six old women reported receiving HRT and exclusion of those women would increase the mean value of tendon CSA from 55.20 to 56.01 mm^2. Finally, an age-related increase in tissue dimensions is also evident in other connective tissue structures, such as increased endosteal and periosteal diameter of the proximal femur in connection with decreased bone mass (3), which preserves bone strength and is also inversely related to estradiol levels (1), as is tendon CSA (17).

Muscle architecture and size. The trends found in muscle architecture are consistent with previous results that have found age-related differences in triceps surae muscle architecture (38, 40, 43). Although we found statistically significant differences between old and young only from GM fascicle length, the lack of other age-related differences in muscle architecture is supported by the findings of Karamanidis and Arampatzis (23, 24). Similar normalized fascicle lengths in GM and SOL between young and old suggest similar capability for muscle shortening and thus similar working range of triceps surae muscles between these groups. Muscle size was significantly lower in old compared with young as indicated by lower muscle thickness in SOL and in GM and lower gastrocnemius CSA. In general it seems that the muscle architecture of triceps surae in older subjects in this study was quite well preserved despite marked changes in muscle force and size. In conjunction with previous literature, our findings suggest that there is a trend for decreased pennation angle and muscle fascicle length with aging but these changes are necessary to maintain functionality of the muscle as muscle size decreases. Discrepancy in previous findings is probably caused by differences in subjects groups. The old subjects in this study were all living independently and attending regularly in social activities and thus do not represent the most frail elderly people.

Achilles tendon mechanical properties. Our findings of age-related differences in Achilles tendon mechanical properties are in accordance with the studies that measured Achilles tendon elongation as a displacement of GM myotendinous junction (39, 44). The studies that have reported similar Achilles tendon properties in old and young subjects have measured the elongation as the elongation of outer tendon and aponeurosis (23, 25). This methodological difference can be the factor causing this discrepancy. Two studies have examined age-related differences in patella tendon properties measuring only the outer tendon (9, 11), and both fail to find differences between old and young in tendon mechanical properties. It is possible that Achilles and patella tendon respond to aging differently as they are morphologically and functionally different. We admit that SD in mean values of hysteresis was large although similar than previously reported (13, 31), causing statistical power to detect group differences to be low. The differences in group means were not large enough to have any real life significance, and thus it can be concluded that the hysteresis values were similar between age groups and sexes. The mean hysteresis for the whole population was 2.7%, which is smaller than reported previously in vivo (13, 19, 31, 55). We did not observe a toe region, and the tendon force-elongation relationship was linear in the region where tendon properties were calculated (10–80% MVC, $r = 0.999$). This was probably due to initial force acting on the Achilles tendon in the seated position and the fact that we excluded the lowest 10% of MVC force from the stiffness and Young's modulus analysis. The relatively small values of hysteresis may thus be due to the fact that the crimped pattern of collagen fibers was already straightened in this joint configuration. In this experimental setting, the Achilles tendon acted almost totally elastically without a viscous component that would increase hysteresis.

When we compared young and old individuals with similar force production capacity we found that tendon stiffness was similar. We also found that tendon stiffness and Young's modulus correlated with muscle strength which has been previously shown in young (54). These findings indicate that aging does not affect the tendon's ability to adjust its mechanical properties to the requirements set by the muscle, but it may be that the strategy for the adjustment is changed due to aging, as tendon CSA was higher in old subjects. Increasing tendon CSA does not seem to be the mechanism for relatively fast adaptation of mechanical properties (28, 52) but might be involved in habitual loading (12, 27) and aging. Finally, lower stress and strain in old compared with young subjects at similar relative force levels may decrease the likelihood of tendon injury in old individuals.

Implications of different tendon properties between young and old. We hypothesize that in low-loading situations such as in walking the lower Achilles tendon stiffness in old individuals allows more elastic energy utilization, muscle fascicles to operate closer to the optimal length and slower speed of fascicle length change when triceps surae muscles are active than if they had similar tendon stiffness as young. Since the amount of elastic energy stored in tendon is $E = 1/2\, kx^2$ (where k is stiffness and x is elongation), the tendon with smaller stiffness loaded with equal force will store more energy. The amount of energy the tendon releases at unloading is dependent on hysteresis and there is no evidence that hysteresis is different between old and young. It has been estimated that in young, muscle fascicles of GM work at slightly longer length than optimal in walking (5, 20), and it could be that the lower Achilles tendon stiffness in old individuals allows muscle fascicles to shorten the amount needed to reach optimal length. In gastrocnemius lateralis, it has been shown that in walking when the muscle is active, fascicle length remains unchanged in older subjects and lengthens in younger subjects (37). Slower contraction velocity is beneficial for the force generation according to the force-velocity relationship. These hypotheses are logical since older individual are likely to use only walking for ambulation and young individuals are more likely

to use both walking and running and the differences in tendon properties would reflect adaptations to the tasks of daily living. Further studies are needed to verify these hypotheses.

Methodological aspects. One of the strengths of this study is that the sample size is the largest to date for studies that have measured tendon properties in vivo. This is important for group comparisons of measures that have large individual variation. State-of-the-art methods were also used to measure Achilles tendon properties. The method combines ultrasonography, motion analysis, heel movement measurement, and force measurement to obtain tendon force-elongation data. A similar method was recently suggested for measuring tendon properties in vivo (21). This method accounts for possible probe movement or heel movement during contractions, which can result in 40% and 30% overestimations of tendon elongation, respectively (21, 33). Strain rate was also matched between young and old subjects to prevent this parameter from influencing tendon properties (46).

Calculation of Achilles tendon force is problematic because not all the plantarflexion force is transmitted through the Achilles tendon, and coactivation of dorsiflexion muscles would decrease measured plantarflexion force and thus calculated Achilles tendon force. Estimation of the proportion of force that is transmitted through the Achilles tendon is difficult because of possible individual variations in plantar flexor activation strategies (14). In this study there was no attempt to correct for coactivation of tibialis anterior or other muscles for several reasons. In a study conducted in our lab coactivation was always less than 5% during isometric plantarflexion (48). Further, the correction of coactivation-induced force relies on the assumption that force and surface EMG are linearly related, which may not be the case (30). Several muscles contribute to ankle joint torque, and it is not possible to measure the activity of them all. We used relatively fast contractions for the measurements of tendon properties and thus we could not average EMG activity over a sufficiently long time period to obtain reliable measures of muscle activity. Finally, correction of the coactivation of muscles requires measurement of force-EMG relationship and it can be assumed that there is also coactivation in several muscles when trying to measure this relationship. Besides the beforementioned facts tendon force calculation relies on knowledge of the lever arms' lengths. The joint rotations observed in this study would cause only negligible changes in Achilles tendon lever arm length (29) and foot lever arm length was confirmed in a pilot study using plantar pressure measurement (Pedar-X, Novel). Thus constant lever arm lengths were considered sufficient approximation for this study.

Future studies and conclusions. Muscle and tendon interaction and the effects of tendon properties on muscle function warrant more attention. In particular it would be interesting to investigate how changes in tendon properties due to training or aging relate to muscle function and economy in different types of movement and in different age groups. In future studies it would also be interesting to examine whether tendon adaptation mechanisms are different between young and old people and to clarify why aging seems to be related to an increase in tendon dimensions.

In conclusion, we showed that there can be substantial age-related differences in tendon properties with only minor differences in muscle architecture. This will probably have effects on the muscle-tendon complex function between young and old people in activities requiring approximately similar force production but not in activities where similar muscle forces are used in relation to the muscles force production capacity. We suggest that discrepant finding for age-related differences in tendon are due to methodological differences and for muscle architecture possible differences in subject groups. It seems that muscle architecture or tendon properties are not responsible for functional deficit in older people. Muscle size and intrinsic properties of muscle tissue are likely to be the main causes of lowered functional capacity. Although it was shown that Achilles tendon stiffness and Young's modulus are lower in older participants, it may be that tendon tissue is optimized to functional requirements rather than deteriorated. The current results suggest that tendon mechanical properties are matched to the force producing capacity of the muscle and adapt to loading rather than the effects of aging. Tendon stiffness was shown to be strength dependent but age independent. In the case of Achilles tendon, changes in tendon properties with aging may actually enhance the function of the muscle-tendon complex in low-loading conditions such as walking.

GRANTS

We acknowledge the support from the EC FP7 Collaborative Project MYOAGE (GA-223576).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: L.S., S.S., and T.F. conception and design of research; L.S. performed experiments; L.S., J.P., and N.J.C. analyzed data; L.S. interpreted results of experiments; L.S. prepared figures; L.S. drafted manuscript; L.S., J.P., N.J.C., S.S., and T.F. approved final version of manuscript; J.P., N.J.C., S.S., and T.F. edited and revised manuscript.

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II

PLANTARFLEXOR MUSCLE-TENDON PROPERTIES ARE ASSOCIATED WITH MOBILITY IN HEALTHY OLDER ADULTS

by

Stenroth L, Sillanpää E, McPhee JS, Narici MV, Gapaeyeva H, Pääsuke M, Barnouin Y, Hogrel J-Y, Butler-Browne G, Biljsma A, Meskers CGM, Maier AB, Finni T & Sipilä S, 2015

The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 70(8), 996-1002
http://dx.doi.org/10.1093/gerona/glv011

III

TRICEPS SURAE MUSCLE-TENDON PROPERTIES IN OLDER ENDURANCE- AND SPRINT-TRAINED ATHLETES

by

Stenroth L, Cronin NJ, Peltonen J, Korhonen MT, Sipilä S & Finni T, 2015

Journal of Applied Physiology 120, 63-69

J Appl Physiol 120: 63–69, 2016.
First published October 22, 2015; doi:10.1152/japplphysiol.00511.2015.

Triceps surae muscle-tendon properties in older endurance- and sprint-trained athletes

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[1]*Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyvaskyla, Jyvaskyla, Finland; and* [2]*Gerontology Research Center and Department of Health Sciences, University of Jyvaskyla, Jyvaskyla, Finland*

Submitted 17 June 2015; accepted in final form 19 October 2015

Stenroth L, Cronin NJ, Peltonen J, Korhonen MT, Sipilä S, Finni T. Triceps surae muscle-tendon properties in older endurance- and sprint-trained athletes. *J Appl Physiol* 120: 63–69, 2016. First published October 22, 2015; doi:10.1152/japplphysiol.00511.2015.— Previous studies have shown that aging is associated with alterations in muscle architecture and tendon properties (Morse CI, Thom JM, Birch KM, Narici MV. *Acta Physiol Scand* 183: 291–298, 2005; Narici MV, Maganaris CN, Reeves ND, Capodaglio P. *J Appl Physiol* 95: 2229–2234, 2003; Stenroth L, Peltonen J, Cronin NJ, Sipila S, Finni T. *J Appl Physiol* 113: 1537–1544, 2012). However, the possible influence of different types of regular exercise loading on muscle architecture and tendon properties in older adults is poorly understood. To address this, triceps surae muscle-tendon properties were examined in older male endurance (OE, $n = 10$, age = 74.0 ± 2.8 yr) and sprint runners (OS, $n = 10$, age = 74.4 ± 2.8 yr), with an average of 42 yr of regular training experience, and compared with age-matched [older control (OC), $n = 33$, age = 74.8 ± 3.6 yr] and young untrained controls (YC, $n = 18$, age = 23.7 ± 2.0 yr). Compared with YC, Achilles tendon cross-sectional area (CSA) was 22% ($P = 0.022$), 45% ($P = 0.001$), and 71% ($P < 0.001$) larger in OC, OE, and OS, respectively. Among older groups, OS had significantly larger tendon CSA compared with OC ($P = 0.033$). No significant between-group differences were observed in Achilles tendon stiffness. In older groups, Young's modulus was 31-44%, and maximal tendon stress 44–55% lower, than in YC ($P \leq 0.001$). OE showed shorter soleus fascicle length than both OC ($P < 0.05$) and YC ($P < 0.05$). These data suggest that long-term running does not counteract the previously reported age-related increase in tendon CSA, but, instead, may have an additive effect. The greatest Achilles tendon CSA was observed in OS followed by OE and OC, suggesting that adaptation to running exercise is loading intensity dependent. Achilles tendon stiffness was maintained in older groups, even though all older groups displayed larger tendon CSA and lower tendon Young's modulus. Shorter soleus muscle fascicles in OE runners may be an adaptation to life-long endurance running.

Achilles tendon; mechanical properties; muscle architecture; aging; exercise

LOSS OF MUSCLE FUNCTION WITH aging is associated with physical limitations and disability (40). Decline in muscle mass is undoubtedly an important contributor to the deterioration in muscle function with aging (16). However, longitudinal studies have shown a clear dissociation in loss of muscle function and cross-sectional area or mass with aging (9, 17), suggesting that other factors may also contribute to the age-related loss of muscle function. Muscle architecture and tendon mechanical properties greatly affect muscle performance (28, 51) and have been found to differ between young and old sedentary adults (36, 37, 44). Thus age-related alterations in muscle architecture and tendon mechanical properties may partially explain the loss of muscle performance with age that occurs at a disproportionally faster rate than the decline in muscle mass.

Regular exercise is a key aspect supporting healthy aging. Indeed, it has been suggested that older athletes provide a model of exceptionally successful biological aging (46). For example, previous studies have shown that aged athletes with systematic exercise training habits exhibit much better cardiorespiratory, metabolic, and bone health than their less active counterparts (22, 49). Regular exercise training, especially strength and sprint exercise, also helps to maintain muscle mass, function (21, 52), and composition (43), thus counteracting the age-related decline in functional performance typically observed in normal populations (9, 39).

Despite several known beneficial effects of regular exercise on the musculoskeletal system in old age, little is known about the effects of regular participation in planned exercise on muscle architecture and tendon properties in older adults. Two previous studies have compared untrained older adults to older endurance runners. First, Karamanidis and Arampatzis (19) found that muscle architecture and tendon stiffness in medial gastrocnemius and vastus lateralis were largely similar in older endurance runners compared with untrained older adults. The only significant difference was greater medial gastrocnemius pennation angle in endurance runners. Second, Couppé et al. (7) recently found that older endurance runners had a greater patella tendon cross-sectional area but similar tendon stiffness compared with untrained peers. These previous studies were conducted on endurance runners, and thus knowledge of the long-term effects of different types of exercise loading on muscle architecture and tendon properties in older adults is missing.

Therefore, the aim of this study was to examine the association between different types of life-long exercise and muscle-tendon properties by comparing muscle architecture and tendon properties in older sprint and endurance runners to both age-matched and young untrained adults. Triceps surae muscles were studied because of their important role in locomotion and because they exhibit the greatest functional limitation of all lower limb muscle groups in older adults during locomotion (24). Endurance running provides a model of high-volume and moderate-intensity loading, while sprint running represents a model of low-volume but high-intensity loading of triceps surae muscles. The hypothesis was that older athletes with a life-long regular running background would exhibit muscle fascicle length, pennation angle, muscle size, muscle strength, and tendon mechanical properties in the triceps surae muscle

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group that are more similar to those of young adults than untrained older adults. In addition, based on previous cross-sectional studies conducted in young adults (1, 2), it was hypothesized that sprint-trained older athletes would be stronger, have stiffer Achilles tendons, lower pennation angle, and longer muscle fascicles compared with endurance-trained older athletes.

MATERIALS AND METHODS

Subjects. Male subjects were recruited in two age categories, one from 18 to 30 yr old [untrained young controls (YC), $n = 18$] and the other from 70 to 80 yr old. The older cohort was recruited in three groups: untrained older controls (OC, $n = 33$), older athletes competing in endurance running events (OE, $n = 10$) and older athletes competing in sprint running events (OS, $n = 10$).

Untrained YC and OC groups were part of a Europe-wide collaborative study called MyoAge (34) and included in the present study to represent general populations of healthy young and older adults. We defined untrained as a person who may be recreationally active but is not training for, or participating in, competitive sport. YC were recruited from among university students using study advertisements via e-mail and bulletin boards. We excluded those who studied sport sciences, as well as competitive athletes. OC were recruited from the University of the Third Age or from weekly community meetings of retired people. The aim was to recruit healthy older people who were socially active and free from comorbidity. Using telephone interviews, an equal number of sedentary and physically active (competitive athletes excluded) older subjects were recruited to obtain a representative sample of older people with varying physical activity levels. Sedentariness was defined as exercising for fitness and health one or fewer times per week. Physically active was defined as exercise three or more times per week (30 min or more with intensity sufficient to cause sweating or breathlessness). Results (44) and more detailed description of the recruitment (34) of YC and OC have been presented earlier.

Older athletes were recruited among the participants of the World Master Athletics Indoor Championships held in Jyväskylä, Finland in 2012. Twenty male athletes were recruited based on the events in which they participated during the championships. Ten subjects were recruited from sprint running events (60 m, 60 m hurdles, 200 m, 400 m) and 10 were recruited from endurance running events (3 km, half marathon, and 8 km cross country running). Some subjects in the OS and OE groups participated in several sprint or endurance running events, respectively. Mean results of the subjects competing in the championships were as follows: 60 m, 9.13 ± 0.48 s ($n = 8$); 60-m hurdles, 10.15 s ($n = 1$); 200 m, 30.64 ± 1.97 s ($n = 7$); 400 m, 1:13 ± 8 min:s ($n = 3$); 3,000 m, 13:48 ± 60 min:s ($n = 4$); half marathon, 1:43:37 ± 11:34 h:min:s ($n = 5$); and 8 km cross country running, 44:19 ± 6:25 min:s ($n = 7$). These results correspond to 8, 1, 11, 16, 22, and 16% slower than the world record times for 75-yr-old men in 60 m, 60-m hurdles, 200 m, 400 m, 3,000 m, and half marathon, respectively. Thus the participating subjects can be considered to be highly competitive athletes.

Subject exclusion criteria were Achilles tendon pain, history of Achilles tendon rupture or surgery, pain in calf muscles during measurements, neurological and progressive severe illnesses, insulin-treated diabetes, fracture within the previous year, immobilization for 1 wk during the last 3 mo, daily use of painkillers, use of immunosuppressive drugs or anticoagulants, or severe visual or hearing impairment.

The ethics committee of the Central Finland Health Care District approved the study. All participants signed an informed consent before participating in the study, and measurements were conducted according to the standards set by the latest revision of the Declaration of Helsinki.

Measurements. Training characteristics of OE and OS groups were assessed with self-reported questionnaire. The athletes were asked about their training history (yr), overall training volume (h/wk), and amount of endurance (km/wk), sprint (sessions/wk), and strength training (sessions/wk) in their current normal training routines.

Laboratory measurements included assessment of triceps surae muscle architecture and size and Achilles tendon cross-sectional area and mechanical properties. The measurement procedures have been previously described in detail (44), but are briefly described below.

For the measurements of Achilles tendon and both gastrocnemius and soleus muscle architecture and size at rest, the subjects were lying prone facing down with ankle angle at 90°. Tendon cross-sectional area (mm^2) was measured from a B-mode ultrasound image taken 4 cm proximal from the proximal border of the calcaneal tubercle where the free Achilles tendon typically reaches its smallest cross-sectional area (38). Body mass normalized tendon cross-sectional area was calculated by dividing cross-sectional area by body $mass^{2/3}$ (20). Muscle architecture from medial gastrocnemius and soleus muscles was assessed from ultrasound images taken at 50% of medial gastrocnemius length and mid-muscle belly in the medial-lateral direction. Fascicle length (mm), pennation angle (°), and muscle thickness (mm) were measured from the images. To take into account between-subject differences in stature, fascicle length was normalized to tibia length. The combined anatomical cross-sectional area (cm^2) of medial and lateral gastrocnemius was measured from a panoramic B-mode ultrasound image taken at 50% of medial gastrocnemius length as a measure of the size of the gastrocnemius muscles. All measurements from ultrasound images were taken twice using an open-source computer program (ImageJ 1.44b, National Institutes of Health), and the mean was used for subsequent data analysis.

For the measurement of Achilles tendon mechanical properties, the subjects were seated in a custom-built dynamometer with ankle angle at 90° and knee fully extended and hip at 60° of flexion (full extension 0°). After a standardized warm-up, three maximal voluntary contractions (MVC) lasting ~3 s were performed with strong verbal encouragement to measure plantar flexion strength (Nm). The highest value obtained during MVC trials was used for subsequent analysis. Warm-up and plantar flexion MVCs served to precondition the tendon before the measurement of tendon mechanical properties (30). Achilles tendon mechanical properties were measured from several isometric plantar flexion contractions up to a force level of 80% of MVC. Tendon force was calculated by multiplying measured reaction force by the ratio between Achilles tendon moment arm length and moment arm of the reaction force. Achilles tendon moment arm was defined as the distance from the center of the Achilles tendon to the outermost tip of the medial malleolus in the sagittal plane measured using a ruler. The moment arm of the reaction force around the ankle joint was defined as the sagittal plane distance between the outermost tip of the medial malleolus and the head of the first metatarsal. Achilles tendon elongation (mm) was defined as the change in the distance between the proximal border of the calcaneal tubercle and the medial gastrocnemius muscle-tendon junction. Changes in the location of the calcaneal tubercle in the laboratory coordinate system were measured using a potentiometer that measures heel lift from the dynamometer footplate. Medial gastrocnemius muscle-tendon junction location in the laboratory coordinate system was measured with a combination of B-mode ultrasonography and motion analysis. Ultrasound images of the muscle-tendon junction were collected at 70 Hz, and the location of the muscle tendon junction within the image was defined by automatic tracking software (32). The location of the muscle-tendon junction was converted to the laboratory coordinate system using video-based motion capture of the ultrasound probe. Two parameters that describe tendon mechanical properties were calculated, tendon stiffness (N/mm) and Young's modulus (GPa). Tendon stiffness characterizes mechanical properties of the tendon and is defined as the slope of the linear portion of the tendon force-elongation curve. We calculated tendon stiffness as a linear fit to force-elongation data from

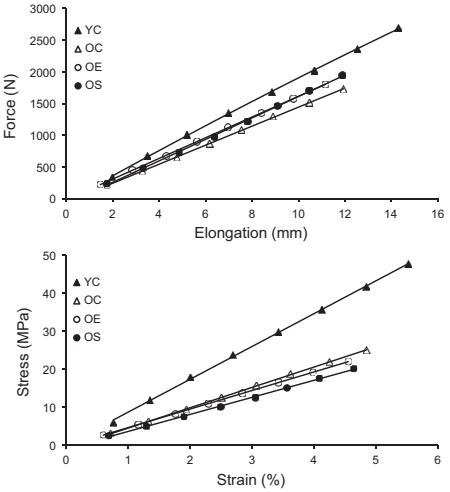

Fig. 1. Mean Achilles tendon force-elongation (*top*) and stress-strain (*bottom*) relationships for young controls (YC), older controls (OC), older endurance runners (OE), and older sprint runners (OS). Lines are linear fits and represent Achilles tendon stiffness (*top*) and Young's modulus (*bottom*). Values are calculated at 10% maximal voluntary contraction increments from 10 to 80% maximal voluntary contraction. SDs are omitted for clarity.

10 to 80% MVC force, as the curves were almost perfectly linear in this region (Fig. 1, $r^2 = 0.999$ from linear fits to average force-elongation curves). Tendon Young's modulus is the slope of the linear portion of the tendon stress-strain curve and represents tendon stiffness normalized to tendon dimensions. Young's modulus describes the mechanical properties of the material from which a tendon is composed. To derive Young's modulus, Achilles tendon stress (Pa) was calculated by dividing Achilles tendon force (N) by tendon cross-sectional area (m^2), and strain (%) was calculated by dividing elongation (mm) by initial tendon length (mm) multiplied by 100. Young's modulus was calculated as a linear fit to force-elongation data from 10 to 80% MVC force.

Statistical analyses. Due to inadequate image quality, soleus muscle architecture data were excluded for two subjects from the OS group and three from the OC group, whereas medial gastrocnemius muscle architecture data were excluded for one subject from OE, and gastrocnemius cross-sectional area data from one subject from OC. Data were first checked for normality with the Shapiro-Wilk test and for homogeneity of variance with Levene's test. Differences in muscle and tendon properties between the groups were tested using single-factor analysis of variance and Tukey-Kramer post hoc test. Games-Howell post hoc test was used when inhomogeneous variances between the groups were observed, and Kruskal-Wallis test with Bonferroni correction for nonnormally distributed variables. Differences in training characteristics between OE and OS were tested using Mann-Whitney *U*-test. The level of statistical significance was set at $\alpha = 0.05$ for all tests. Statistical analyses were performed using IBM SPSS Statistics (version 20.0.0.2). Standardized mean differences between YC and groups of older adults were calculated for main results of the study (see Tables 2 and 3) as a measure of effect sizes using Hedges' g, including a correction for small sample bias (12).

RESULTS

Subject characteristics and training status for the older athletes are reported in Table 1. Older adults in the three different groups were matched for age, height, and body mass. YC were significantly taller than OC ($P < 0.001$). OC had significantly greater body mass index compared with YC ($P = 0.006$) and OE ($P = 0.009$). Significantly lower plantar flexion strength was found in OC (34%, $P = 0.001$) and OE (42%, $P < 0.001$) compared with YC, but not in OS compared with YC ($P = 0.077$). OE and OS groups did not differ in years of training, hours of training per week, or number of strength training sessions per week. Endurance training measured in distance was eight times greater in OE compared with OS ($P < 0.001$), and OS did three times more sprint training sessions per week than OE ($P = 0.006$).

Achilles tendon cross-sectional area was 22, 45, and 71% larger in OC ($P = 0.022$), OE ($P = 0.001$), and OS ($P < 0.001$) compared with YC, respectively (Table 2). Tendon cross-sectional area in OS was significantly larger than in OC ($P = 0.033$). Body mass-normalized tendon cross-sectional area yielded similar results to the unnormalized values. No statistically significant differences were observed between the groups in Achilles tendon stiffness (Fig. 1), but Young's modulus was 31, 35, and 44% smaller in OC ($P < 0.001$), OE ($P = 0.001$), and OS ($P < 0.001$) compared with YC, respectively. Maximal tendon force during MVC was significantly lower in OC (35%, $P < 0.001$) and OE (38%, $P < 0.001$), but not in OS ($P = 0.156$), compared with YC. Average tendon stress during MVC was greater in YC than the older groups ($P < 0.001$). Tendon elongation at 80% MVC was significantly greater in YC compared with OC ($P = 0.014$), but the differ-

Table 1. *Subject characteristics and training status of older athletes*

	YC	OC	OE	OS
Subjects, no.	18	33	10	10
Age, yr	23.7 ± 2.0	74.8 ± 3.6*	74.0 ± 2.8*	74.4 ± 2.8*
Height, cm	181 ± 6	173 ± 5*	175 ± 7	176 ± 7
Body mass, kg	75.4 ± 9.0	76.1 ± 7.7	69.9 ± 6.9	74.3 ± 7.1
BMI, kg/m^2	23.1 ± 2.6	25.4 ± 2.4*†	22.7 ± 1.6	24.1 ± 1.9
Plantar flexion strength, Nm	199 ± 56	132 ± 21*	116 ± 25*	153 ± 39
Length of training, yr			39.4 ± 20.9	44.7 ± 19.7
Training per week, h			6.8 ± 3.3	6.2 ± 2.6
Endurance training per week, km			55.2 ± 8.8	6.5 ± 2.8†
Sprint training sessions per week, no.			0.8 ± 0.7	2.3 ± 1.2†
Strength training sessions per week, no.			0.5 ± 0.2	0.9 ± 0.2

Values are means ± SD. BMI, body mass index; YC, young controls; OC, older controls; OE, older endurance runners; OS, older sprint runners. Significantly different from *YC and †OE: $P < 0.01$.

Table 2. *Achilles tendon cross-sectional area and mechanical properties*

	YC	OC	OE	OS
Cross-sectional area, mm^2	56.5 ± 9.6	69.0 ± 12.2 (−1.05)*‡	82.0 ± 19.8 (−1.69)†	96.5 ± 24.9 (−2.26)†
Stiffness, N/mm	186 ± 37	164 ± 47 (0.49)	172 ± 39 (0.34)	166 ± 35 (0.51)
Young's modulus, GPa	0.86 ± 0.20	0.59 ± 0.17 (1.46)†	0.56 ± 0.22 (1.40)†	0.48 ± 0.19 (1.85)†
Maximum tendon force, kN	3.4 ± 0.9	2.2 ± 0.6 (1.58)†	2.1 ± 0.4 (1.64)†	2.6 ± 0.8 (0.80)
Maximum tendon stress, MPa	59.3 ± 14.9	33.1 ± 9.0 (2.22)†	26.5 ± 8.3 (2.36)†	30.1 ± 14.3 (1.86)†
Elongation at 80% MVC, mm	14.3 ± 2.5	11.9 ± 6.4 (0.42)*	11.2 ± 4.4 (0.88)	11.9 ± 4.2 (0.70)
Strain at 80% MVC, %	5.6 ± 1.5	4.8 ± 2.2 (0.42)	4.5 ± 1.8 (0.66)	4.7 ± 1.7 (0.56)

Values are means ± SD (with effect size compared with YC in parentheses). MVC, maximal voluntary contraction. Significantly different from YC: *$P <$ 0.05, †$P <$ 0.01. ‡Significantly different from OS, $P <$ 0.05.

ence did not reach statistical significance in OE ($P = 0.114$) or OS ($P = 0.352$). However, effect sizes between YC and OE and OS were greater than the effect size between YC and OC. The groups did not differ significantly in tendon strain at 80% MVC.

Results of soleus and gastrocnemius muscle architecture and size, as well as plantar flexion muscle strength, are presented in Table 3. Soleus fascicle length was significantly shorter in OE compared with YC (absolute $P = 0.014$, normalized $P = 0.002$) and also compared with OC (absolute $P = 0.047$, normalized $P < 0.001$). No significant differences were found in soleus pennation angle or muscle thickness. Medial gastrocnemius fascicle length and pennation angle did not differ between the groups. In OC, medial gastrocnemius muscle thickness was significantly smaller in contrast to YC ($P = 0.043$) and gastrocnemius cross-sectional area was significantly smaller in contrast to YC ($P = 0.011$) and OS ($P = 0.011$).

DISCUSSION

We examined selected triceps surae muscle-tendon properties of two differently trained groups of older athletes with an average of 42 yr of regular running training and compared them to untrained age-matched older and young adults. The main findings of the study were that Achilles tendon cross-sectional area was significantly larger in all older adult groups than young adults, and in older sprinters compared with age-matched untrained older adults, whereas there were no statistically significant group differences in Achilles tendon stiffness. The greater tendon cross-sectional area was also reflected in tendon Young's modulus and tendon average tensile stress during maximal isometric force production, both of which were significantly lower in all older groups compared with young untrained adults. Only minor differences were observed in triceps surae muscle architecture, the most important being significantly shorter fascicle length in soleus muscle in older endurance runners. The present study adds new insight into possible effects of exercise loading on muscle and tendon structure and function in older age. The novelty of the present study is that measurements of triceps surae muscle architecture and Achilles tendon properties were made from top-level older athletes that included both endurance and sprint runners.

Achilles tendon properties. To the best of our knowledge, this is the first study to show greater Achilles tendon cross-sectional area in older adults with a regular exercise training background. Contradicting our hypothesis, the results suggest that long-term exercise did not counteract the age-related increase in Achilles tendon cross-sectional area. Previous cross-sectional studies suggest that Achilles tendon cross-sectional area increases in response to both long-term exercise loading (20, 33) and normal aging (31, 44). The present results suggest that the Achilles tendon responds to regular loading by increasing cross-sectional area in an intensity-dependent manner. Moreover, the increase in cross-sectional area appears to be additive to the increase due to normal aging. This finding supports recent findings by Couppé et al. (7), who showed that regular endurance running was associated with larger patella tendon cross-sectional area in both young and older adults.

A possible explanation for aging and exercise training to be associated with larger tendon cross-sectional area is that tendon hypertrophy is needed to compensate for an age-related decrease in mechanical properties of the tendon collagen structure. Another possible explanation is that greater tendon cross-sectional area in older adults is observed as a consequence of intratendinous accumulation of lipids or water. These two possible mechanisms are not exclusive and could together explain the observed results. The following paragraphs introduce these proposed explanations in more detail.

Table 3. *Muscle architecture and size*

	YC	OC	OE	OS
Soleus fascicle length, mm	40.6 ± 8.8	38.6 ± 7.6 (0.24)^c	31.2 ± 3.9 (1.18)^a	35.3 ± 8.3 (0.57)
Normalized soleus fascicle length, mm/mm	0.102 ± 0.021	0.100 ± 0.021 (0.11)^d	0.073 ± 0.008 (1.55)^b	0.083 ± 0.022 (0.83)
Soleus pennation angle, °	21.0 ± 5.7	21.2 ± 4.0 (−0.05)	23.7 ± 5.3 (−0.46)	21.6 ± 8.3 (−0.08)
Soleus thickness, mm	14.3 ± 2.6	13.1 ± 2.7 (0.44)	13.4 ± 2.7 (0.33)	12.8 ± 3.7 (0.49)
MG fascicle length, mm	47.7 ± 6.6	45.0 ± 7.6 (0.35)	45.3 ± 6.5 (0.34)	47.7 ± 7.0 (0.00)
Normalized MG fascicle length, mm/mm	0.121 ± 0.018	0.117 ± 0.022 (0.17)	0.108 ± 0.015 (0.71)	0.111 ± 0.021 (0.46)
MG pennation angle, °	24.8 ± 4.0	24.4 ± 4.2 (0.09)	23.3 ± 4.8 (0.34)	24.1 ± 3.5 (0.18)
MG thickness, mm	20.1 ± 2.5	17.7 ± 3.2 (0.77)^a	17.2 ± 3.6 (0.94)	18.6 ± 2.7 (0.55)
Gastrocnemius cross-sectional area, cm^2	24.2 ± 4.5	20.1 ± 4.5 (0.89)^{a,e}	20.9 ± 3.4 (0.73)	25.1 ± 4.4 (−0.19)

Values are means ± SD (with effect size compared with YC in parentheses). MG, medial gastrocnemius. Significantly different from YC: $^a P < 0.05$, $^b P <$ 0.01. Significantly different from OE: $^c P < 0.05$, $^d P < 0.01$. ^{e}Significantly different from OS: $P < 0.05$.

In animal models, aging has been linked with an increase in type V collagen and a greater proportion of small collagen fibrils, which probably contribute to concurrently observed reduced ultimate tensile stress (10, 48). Greater tendon cross-sectional area in older adults could be due to a necessary adaptation to reduce maximal tendon stress to safe levels for older tendons that possibly have reduced ultimate tensile stress. To reduce the stress to a safe level, cross-sectional area must be proportional to maximal force acting on the tendon, thus explaining the greater cross-sectional area in older sprint runners compared with older untrained adults observed in the present study.

Greater tendon cross-sectional area in older adults could also serve to maintain sufficient stiffness, which could be important both for protecting the tendon from strain-induced damage and for muscle function. A possible age-related reduction in stiffness of tendon collagen structure may be partly compensated by an age-related increase in collagen cross-links, especially in advanced glycation end-product cross-links (6), which stabilize collagen structure and may increase tendon stiffness. Life-long endurance running has been shown to be associated with lower advanced glycation end product cross-link density (7). If older athletes in the present study had a lower density of collagen cross-links, this could explain the requirement for older athletes to have even greater tendon cross-sectional area compared with untrained older adults, to maintain tendon stiffness with aging.

Based on current knowledge of tendon adaptation, loading intensity is the main factor determining adaptations in tendon mechanical properties (5). Thus it seems unlikely that sprint-trained older athletes would have the lowest Achilles tendon Young's modulus among the groups in the present study. A possible explanation could be that larger tendon cross-sectional area in older adults is not an adaptation to lowered tendon Young's modulus. Instead it could be due to accumulation of tendon subcomponents that do not markedly affect tendon mechanical behavior. These could include extracellular lipid deposits and proteoglycans and glycosaminoglycans that attract water. Extracellular lipid deposits within tendon have been associated with aging (14), and this could be common to all older adults, irrespective of exercise training. On the other hand, production of proteoglycans and glycosaminoglycans could be increased with exercise training-induced tendon loading (15). This would explain the observed lower Young's modulus and stress of the tendon in older adults in the present study and also explains why greater tendon cross-sectional area was not related to greater tendon stiffness.

Within- and between-operator reliability of Achilles tendon cross-sectional area measurement using ultrasound imaging has been reported to be good (11, 50). In the present study, duplicate analysis of tendon cross-sectional area images produced intraclass correlation 0.989 and typical error 2.1%. However, validity of tendon cross-sectional area measurement using ultrasound imaging is not known; thus the results should be interpreted with some caution. Future studies should try to replicate the findings of the present study, preferably using magnetic resonance imaging, which allows measurements of tendon cross-sectional area along the whole tendon. More research examining tendon composition and collagen structure in older adults is also warranted to explain the mechanisms behind changes in tendon cross-sectional area.

In contrast to our hypothesis that life-long running would mitigate age-related changes in tendon mechanical properties, we found that Young's modulus was significantly lower in older compared with young adults, irrespective of training status, with no significant differences between the older groups. There were also no significant between-group differences in initial tendon length or tendon stiffness. Thus the lower Young's modulus in older compared with young adults can be attributed mainly to the larger tendon cross-sectional area in older adults.

It should be noted that a toe-region with a lower slope of the tendon force-elongation curve at low forces or stresses was not observed (Fig. 1). We think that the reason for highly linear force-elongation/stress-strain curves is initial force acting on the Achilles tendon at a 90° ankle angle, and the fact that we calculated the curve starting from 10% MVC force. Lack of toe-region has also been previously observed for Achilles tendon in vivo when elongation is measured from the medial gastrocnemius muscle-tendon junction (27), as done in the present study.

To summarize the findings regarding tendon mechanical properties, Young's modulus of the Achilles tendon was significantly lower in older compared with young adults, irrespective of training status. Despite this, Achilles tendon stiffness was conserved in all groups of older adults. Thus the lower muscle strength, greater tendon cross-sectional area, and conserved tendon stiffness resulted in reduced maximal tendon stress and strain in older adults. Reduced tendon stress and strain could be a necessary mechanism to decrease the probability of tendon injury, as aging may decrease tendon fascicle sliding that possibly leads to greater loading of the fascicles themselves (47). A functional consequence of similar Achilles tendon stiffness but lower muscle strength in older compared with young adults is a limited maximal capacity for elastic energy storage and subsequent utilization during locomotion. This may contribute to the reported greater metabolic cost of transport in older compared with young adults (35).

Triceps surae muscle architecture, size, and strength. The present data also suggest that, in general, muscle architecture is not greatly different in older habitual runners in contrast to both untrained older or young adults. Soleus fascicle length was found to be significantly shorter in endurance-trained older adults than young and older untrained adults. Although somewhat speculative, it may be that shorter fascicles observed in long-term endurance runners in the present study are due to adaptation that improves the efficiency of force production in locomotion. Soleus has short muscle fascicles compared with tendon length (51). Consequently, soleus muscle operates mainly as a force rather than a power producer in locomotion (4). Thus, as this muscle does not need to produce large amounts of work, short fascicles may decrease the energy cost of force production due to lower activated muscle volume per unit of force output compared with longer fascicles (29). We recently observed that shorter fascicle length in soleus and gastrocnemius was associated with better mobility in older adults (45), further supporting the suggestion that shorter soleus fascicle length in older endurance runners may be an adaptive response to life-long exercise training.

Another finding of the present study is that long-term endurance running was not associated with greater strength or size of triceps surae muscles compared with untrained older

controls. Plantar flexion strength and maximal tendon force in endurance-trained older adults was significantly lower compared with that in young adults. Moreover, the effect sizes for the difference in gastrocnemius thickness and cross-sectional area were comparable to those between young and older controls, which were also statistically significant. Taken together, these results suggest that endurance running is not a sufficient stimulus for maintenance of muscle mass and size with aging.

In contrast, the present data suggest that high-intensity loading due to sprint training may be an effective stimulus to counteract the age-related decline in both muscle mass and strength in triceps surae muscles. We observed that gastrocnemius muscle cross-sectional area was significantly larger in sprint-trained older adults compared with untrained older controls. In addition, plantar flexion strength and maximal tendon force were not significantly different from those of young controls, with about one-half the effect size as in endurance-trained older adults compared with young controls. These findings are supported by previous studies in young adults in which sprint running but not endurance running was associated with greater muscle strength and size in triceps surae muscles (18, 23). It may be that the beneficial effects of sprint training preferentially target gastrocnemius muscle, which contains more fast-twitch muscle fibers than soleus (13).

Methodological considerations. The strengths of the present study are that the world-class older athletes measured in the present study had a life-long physical activity background and had performed many decades of regular exercise training. In addition, both the trained and untrained older adults were over 70 yr old and thus can be assumed to be affected by primary biological aging.

Limitations of the present study include the cross-sectional study design, which does not allow conclusions about cause-effect relationships that a longitudinal study design may allow. Cross-sectional studies can be affected by selection bias. It is possible that subjects with favorable muscle-tendon properties for endurance or sprint running were more likely to participate in such activities. However, we did not observe differences between older trained and untrained subjects in genetically determined variables such as Achilles tendon moment arm, forefoot length, or Achilles tendon length, all of which are related to running performance (3, 25, 26, 42). This suggests that selection bias caused by genetic predisposition toward favorable musculoskeletal properties for running did not considerably affect our data, although the possibility of selection bias cannot be completely excluded. Another limitation of the present study is the small sample size. However, it was not possible to obtain a larger sample of older athletes from the highest performance level.

Conclusions. The present findings suggest that triceps surae muscle size, architecture, strength, and tendon stiffness are relatively unaffected by long-term running training in older adults. The reason for this finding may be that the triceps surae muscle group is highly loaded in daily activities, and thus training produces only a small relative overload to this muscle group. Considering the unparalleled physical performance of the older athletes in the present study, it appears that the measured triceps surae muscle-tendon properties are not the key determining factors in their physical performance. However, relatively high individual variation in these properties suggests that a well-functioning muscle-tendon unit may be achieved via different combinations of muscle and tendon properties. In addition, it is likely that, in the present study, there were differences between the groups in factors that were not measured but that affect physical performance. These include muscle fiber type, composition, molecular level modifications in contractile proteins, and neural activation (8, 21, 41). To further elucidate the importance of muscle architecture and tendon mechanical properties for physical performance, future studies should investigate how aging and physical loading affect muscle-tendon interaction during locomotion.

In conclusion, our data suggest that long-term physical loading induced by either endurance or sprint running does not have a significant effect on Achilles tendon stiffness in older adults. However, the loading patterns associated with sprint and endurance training in older age both appear to increase Achilles tendon cross-sectional area in an intensity-dependent manner. Furthermore, the present results suggest that sprint running but not endurance running may mitigate age-related loss of muscle mass and strength in triceps surae muscles. On the other hand, endurance training in older age may alter muscle architecture in a way that is beneficial for movement economy.

GRANTS

This work was supported by grants from the Finnish Cultural Foundation to L. Stenroth and by the EC FP7 Collaborative Project MYOAGE (GA-223576).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: L.S., N.J.C., S.S., and T.F. conception and design of research; L.S. performed experiments; L.S. and J.P. analyzed data; L.S. and M.T.K. interpreted results of experiments; L.S. prepared figures; L.S. drafted manuscript; L.S., N.J.C., J.P., M.T.K., S.S., and T.F. edited and revised manuscript; L.S., N.J.C., J.P., M.T.K., S.S., and T.F. approved final version of manuscript.

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IV

SLOWER WALKING SPEED IN OLDER ADULTS IMPROVES TRICEPS SURAE FORCE GENERATION ABILITY

by

Stenroth L, Sipilä S, Finni T, Cronin NJ.

Medicine & Science in Sports & Exercise, doi: 10.1249/MSS.0000000000001065

Medicine & Science IN Sports & Exercise
The Official Journal of the American College of Sports Medicine
www.acsm-msse.org

. . . Published ahead of Print

Slower Walking Speed in Older Men Improves Triceps Surae Force Generation Ability

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Accepted for Publication: 22 July 2016

Medicine & Science in Sports & Exercise, Publish Ahead of Print
DOI: 10.1249/MSS.0000000000001065

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Running title: Why do older adults walk slower?

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This study was supported by grants from the Finnish Cultural Foundation and Emil Aaltonen Foundation to L.S. The authors have no financial or other conflicts of interest to declare. The results of the present study do not constitute endorsement by ACSM.

Abstract

Purpose: Older adults walk slower than young adults but it is not known why. Previous research suggests that ankle plantarflexors may have a crucial role in the reduction of walking speed. The purpose of this study was to investigate age-related differences in triceps surae muscle-tendon function during walking to further investigate the role of plantarflexors in the age-related reduction of walking speed. **Methods:** Medial gastrocnemius and soleus muscle fascicle lengths were measured using ultrasound imaging during walking from 13 young (25±4yrs) men at preferred walking speed, and from 13 older (73±5yrs) men at preferred speed and at the young men's preferred speed. Muscle-tendon unit lengths were calculated from joint kinematics and tendinous tissue lengths were calculated by subtracting muscle lengths from muscle-tendon unit lengths. In addition, ground reaction forces and electromyographic activity of medial gastrocnemius and soleus were measured. **Results:** In both medial gastrocnemius and soleus it was observed that at preferred walking speed, older men used a narrower muscle fascicle operating range and lower shortening velocity at the estimated time of triceps surae peak force generation compared to young men. Fascicles also accounted for a lower proportion of muscle-tendon unit length changes during the stance phase in older compared to young men. Significant differences in triceps surae muscle function were not observed between age groups when compared at matched walking speed. **Conclusions:** In older men, walking at preferred speed allows triceps surae muscles to generate force with more favorable shortening velocity and to enhance use of tendinous tissue elasticity compared to walking at young men's preferred speed. The results suggest that older men may prefer slower walking speeds to compensate for decreased plantarflexor strength.

Keywords: aging, ultrasound, fascicle, tendon, gastrocnemius, soleus

Introduction

Older adults tend to walk slower than young adults. The slowing of preferred walking speed is a well-established feature of aging and although several mechanisms have been proposed to explain the age-related reduction in the preferred walking speed, a consensus has not been reached (29).

Preferred walking speed is a good predictive tool of health-related outcomes in older adults (22). It predicts adverse health outcomes (32), development of mobility disability (1) and mortality (40). Thus, it would be of interest to better understand the causes of the reduction in preferred walking speed with aging.

Reduced ankle plantarflexion peak power in the late push-off phase of walking is a consistent finding in both healthy and mobility limited older compared to young adults (10, 23, 30), and it seems that impairments in plantarflexor force and power generation contribute to a reduction in preferred walking speed in older adults (29). Tendinous tissues (tendon and aponeurosis, TT) elasticity greatly affects muscle function of the main plantarflexor, triceps surae, due to a large tendon to muscle fiber length ratio (41). Using ultrasound imaging, it has been revealed that TT elasticity is utilized in triceps surae to enhance power generation during the push-off phase of walking (11, 21).

Based on previous modeling and experimental studies, there seems to be an optimal TT stiffness to maximize muscle efficiency or power generation in cyclic contractions (24, 27). Several in vivo human studies have linked aging with a decrease in Achilles tendon or triceps surae TT stiffness (9, 20, 28, 34, 38). Thus, age-related changes in triceps surae TT elastic properties may contribute to reduced plantarflexor performance in walking in older adults. In fact, we

showed earlier that 6-minute walking test results positively correlate with Achilles tendon stiffness in older adults (39). Additionally, recent studies using ultrasound imaging and musculoskeletal modeling have started to unfold more complex interactions between Achilles tendon mechanical properties and triceps surae muscle function in which age-related Achilles tendon interfascicular adhesions coupling gastrocnemius and soleus muscle function may contribute to the reduced plantarflexor performance in walking in older adults (15, 16).

Two previous studies have examined differences in triceps surae muscle fascicle behavior between young and older adults in walking using ultrasound imaging. At the preferred walking speed of older adults (1.1 m/s), Mian et al. (31) found that fascicle lengthening contributed less and TT lengthening more to muscle-tendon unit lengthening in older compared to young adults in lateral gastrocnemius, which is consistent with the previously reported age-related decrease in Achilles tendon stiffness. Panizzolo et al. (36) exploited an approach where older adults walked at their preferred speed and in addition at a speed matched to the preferred speed of young adults. The results showed that soleus muscle fascicle length and length changes were similar between the groups when older adults were allowed to walk at their preferred speed. This result suggests adaptation of preferred walking speed in older adults to preserve soleus muscle fascicle mechanical behavior.

This study investigated triceps surae muscle-tendon function in young and older men to gain insight into the mechanisms that may underlie reduced plantarflexor force and power generation in older adults in walking, and therefore possibly also reduced preferred walking speed. The purpose of the study was to compare triceps surae muscle-tendon function (fascicle length and velocity and TT length changes) between young and older men at matched (young men's preferred walking speed) and preferred walking speeds. In addition, triceps surae muscle-tendon

function was compared within older men between the two walking speeds. Based on previous literature we hypothesized that when the groups were compared at matched walking speed, TT in triceps surae muscles would elongate more in older compared to young men due to lower stiffness, leading to greater muscle fascicle shortening amplitude and shortening velocity in older men to compensate for greater tendon stretch. We also hypothesized that when young and older men were compared at preferred walking speeds, there would be no differences in triceps surae muscle-tendon function between the groups.

Methods

Subjects

Thirteen young (YOUNG, 20-31 years old) and 13 older (OLDER, 67-81 years old) men volunteered for the study. The sample size was considered sufficient based on previous study using similar methodology (34) providing approximately 80% statistical power. YOUNG were recruited using email advertisements among university students and OLDER were recruited from local meetings of older people. All subjects were community living and were able to walk without pain or discomfort. The ethics committee of the University of Jyväskylä approved the study and all participants signed an informed consent prior to participation in the study. The study was conducted according to the principles set by the Declaration of Helsinki.

Walking trials

Subjects were asked to walk over a 10-meter force platform at their preferred walking speed. They started walking three meters before and stopped walking three meters after the force platform to ensure that walking speed was stable over the force platform. The trials were timed using photocells at the start and end of the force platform and the average walking speed was

calculated from the measured time. The average walking speed of three consecutive trials with walking speeds within ±5% was defined as preferred walking speed. In addition to the preferred walking speed, OLDER performed trials at a speed that matched the mean preferred speed of YOUNG within ±5%. Therefore, all YOUNG were tested before OLDER. In both preferred and matched speed trials, data were recorded from at least three trials. In addition, all subjects performed one trial with maximal walking speed from which only the time of the trial was recorded.

Muscle fascicle length and pennation angle

Muscle fascicle length and pennation angle of medial gastrocnemius and soleus muscles were simultaneously measured. An ultrasound probe (7.5 MHz linear array probe with 60 mm field width, Telemed, EchoBlaster128, Lithuania) was attached over the medial gastrocnemius muscle visualizing both medial gastrocnemius (MG) and soleus muscles simultaneously (7). Images were collected at 80 Hz and stored on a PC. Muscle fascicle length and orientation and orientation of the aponeurosis between MG and soleus were tracked from the ultrasound images using an automatic tracking algorithm (8) in MATLAB (MathWorks Inc., Natick, MA, US). The algorithm produces the orientation of the tracked anatomical feature (fascicle or aponeurosis) in relation to the probe orientation. This information was used to calculate pennation angle of the fascicles (angle between muscle fascicles and the aponeurosis). Soleus muscle fascicle data from one older subject were not analyzed due to inadequate image quality.

Reference lengths of muscle fascicles were measured in neutral anatomical position (knee extended and ankle angle at 90°) for normalization of the fascicle lengths during walking. The reference lengths were measured three times in a resting condition while subjects were seated in a dynamometer and the mean value was used for normalization. The neutral anatomical position was chosen for reference length measurements since it has been previously used to represent

optimal fascicle length (24), i.e. the plateau region of the sarcomere force-length relationship. Operating length was defined as the normalized fascicle length at a specific instant of time or over a time range, and operating range was defined as the range of normalized fascicle lengths over a given time range.

Maximal voluntary contraction force

Plantarflexion maximal voluntary contraction force was measured at 2000 Hz (Cambridge Electronic Design, Cambridge, UK) in a custom built dynamometer (University of Jyväskylä, Finland) where subjects were seated with knee extended and ankle angle at 90°. After a standardized warm-up, three isometric maximal voluntary contractions lasting approximately 3 seconds were performed. The highest force generation obtained was taken as plantarflexion strength. Force produced against the dynamometer's footplate was chosen as a measure of muscle strength rather than ankle joint moment since it was considered that this measure reflects the maximal capacity for force generation in the push-off phase of walking taking into account possible differences in forefoot length between the subjects.

Muscle-tendon unit and tendon length

During walking, sagittal plane ankle and knee joint angles were measured at 2000 Hz (Cambridge Electronic Design, Cambridge, UK) using custom built electrogoniometers (University of Jyväskylä, Finland) attached over the lateral side of the joints. Muscle-tendon unit (MTU) lengths for both MG and soleus were calculated from the joint kinematics using regression equations defined by Hawkins & Hull (16). Tendinous tissue (TT, including both proximal and distal free tendon and aponeurosis) lengths for both MG and soleus were calculated as $L_{TT} = L_{MTU} - L_{FAS}*\cos(\text{pennation angle})$, where L_{MTU} is MTU length and L_{FAS} is muscle fascicle length (15).

MTU and TT lengths were normalized by dividing them by their corresponding reference lengths, which were determined in the same way as the muscle fascicle reference lengths.

Muscle activity

To determine muscle activity in walking and in MVC tests, electromyography (EMG) was measured from MG and soleus using a telemetric EMG system (Noraxon Inc. Scottsdale, AZ, USA). After shaving and preparing the skin with fine sandpaper and alcohol wipes, two electrodes (Ambu A/S, Ballerup, Denmark) were attached over the skin with 22 mm interelectrode distance according to SENIAM recommendations (17). The data were sampled at 1500 Hz, preamplified and sent wirelessly to an A/D converter (Cambridge Electronic Design, Cambridge, UK). EMG data from one older subject were lost due to technical problems in data collection.

Ground reaction forces

Three dimensional ground reaction forces (GRF) were recorded using two custom built 10-meter long (University of Jyväskylä, Finland) force platforms positioned side by side that allowed reaction forces to be measured separately for both legs. The data were collected at 2000 Hz (Cambridge Electronic Design, Cambridge, UK). GRFs were normalized by dividing the forces by body weight.

Data analysis

Data analyses were performed in MATLAB (MathWorks Inc., Natick, MA, US). All data were interpolated to 1000 Hz and synchronized. EMG data were band-pass filtered between 20 and 450 Hz using a forth order zero-lag Butterworth filter. GRFs were low-pass filtered at 40 Hz and joint kinematics and ultrasound data were low-pass filtered at 15 Hz (forth order zero-lag Butterworth filter). Stride cycles were separated based on vertical ground reaction force data and

interpolated to 1000 data points. Data from each stride were averaged within a subject and the mean data were used in further analysis. An exception to this was EMG data from which root mean squared (RMS) values during the stance and push-off phases were calculated from each stride, normalized to maximal 500 ms RMS obtained during MVC tests and then averaged across strides. For visualization, moving 100 ms RMS windows were used to produce EMG envelopes. To calculate fascicle, MTU and TT velocities, subject's mean data was interpolated back to units of time using the mean duration of the included strides, and velocities were calculated by numerical differentiation of lengths with respect to time.

The start of the push-off phase was identified from the anterior-posterior GRF as the instant at which the force changed from negative to positive. Selected variables were calculated from the whole stance phase (hereafter referred to as stance) or from the push-off phase (hereafter referred to as push-off). TT peak length was used as a surrogate measure of the instant of muscle-tendon unit peak force. TT maximal elongation was calculated as the difference between maximal and minimal length during the whole stride. Strain was calculated by dividing maximal elongation by TT reference length.

The number of strides included in the analysis per subject was 25±7, 28±8 and 23±8 for YOUNG, OLDER preferred speed and OLDER matched speed, respectively. A different number of strides was included from separate trials. To ensure that the reported walking speeds reflected the average stride of each individual, walking speed was calculated as the weighted mean of the trials where the number of included strides from each trial was used as the weights.

Statistical analysis

Data were first checked for normality with Shapiro-Wilk test and for homogeneity of variance with Levene's test. Differences between YOUNG and OLDER were tested using inde-

pendent samples t-test and differences between preferred and matched speed within OLDER were tested using paired samples t-test. For non-normally distributed variables Mann-Whitney U-test and Wilcoxon signed rank test were used for between and within group comparisons, respectively. Associations between preferred walking speed and parameters related to triceps surae muscle-tendon function were tested using Pearson product-moment correlation. Regression analysis was used to test effect of age on the associations. The level of statistical significance was set at $p<0.05$. Statistical analyses were performed using IBM SPSS Statistics (version 20.0.0.2).

Results

Subject characteristics

Sample means of YOUNG and OLDER were relatively similar in height but OLDER were slightly heavier and had higher body mass index compared to the sample means of YOUNG (table 1). OLDER were on average 32% weaker in body weight-normalized plantarflexion strength compared to the YOUNG (1.3 vs 1.9 N/body weight, $p=0.001$). No significant differences were observed between YOUNG and OLDER in fascicle, MTU or TT reference lengths that were used to normalize the data.

Spatio-temporal gait parameters

Spatio-temporal parameters of walking are reported in table 2. The preferred speed was 18% slower in OLDER compared to YOUNG ($p<0.001$) but the matched speed in OLDER was similar to the preferred speed of YOUNG ($p=0.946$). Similarly, other spatio-temporal gait parameters significantly differed between YOUNG and OLDER at preferred speed ($p<0.05$) but not at matched speed ($p>0.05$). Maximal walking speed was 13% slower in OLDER compared to YOUNG (2.01±0.22 vs. 2.31±0.39 m/s, $p=0.024$).

Muscle fascicle lengths

In both YOUNG and OLDER, after initial shortening, MG fascicles were relatively isometric through the first half of stance after which the fascicles shortened during push-off. For soleus, fascicle shortening started earlier, already during the first half of stance. There was also more pronounced lengthening of soleus muscle fascicles during the first half of stance in OLDER, which was not evident in YOUNG (figure 1).

Muscle fascicle operating lengths are shown in figure 2. Both MG and soleus muscles operated at a narrower range in OLDER compared to YOUNG in stance at preferred speed (MG 7.5±2.7 vs 12.0±4.8 % of reference length, p=0.014, soleus 4.9±1.7 vs 8.5±3.9 % of reference length, p=0.007). However, no significant differences were observed between the age groups at matched speeds. Also, operating ranges did not significantly differ between YOUNG and OLDER when calculated from push-off only. In OLDER, increase in walking speed significantly increased the operating range in MG both in whole stance and push-off, and in stance only in soleus (MG stance 7.5±2.7 vs 9.3±3.1 % of reference length, p=0.003, MG push-off 6.6±2.6 vs 7.8±3.1 % of reference length, p=0.016, soleus stance 4.9±1.7 vs 6.5±1.7 % of reference length, p<0.001).

YOUNG and OLDER did not significantly differ in mean fascicle length during stance or push-off or in fascicle lengths at peak TT length. In OLDER, walking speed did not have a significant effect on MG mean fascicle length during stance, MG or soleus mean fascicle length during push-off or MG or soleus fascicle length at peak TT length.

Muscle-tendon unit and tendinous tissues lengths

MTU lengths were derived from joint angles and thus joint angles are not separately reported. MTU peak length and length at toe-off were significantly longer in OLDER compared to YOUNG in both muscles at preferred speed (peak length: MG 1.015±0.009 vs 1.009±0.010, p=0.044, soleus 1.023±0.011 vs 1.016±0.014, p=0.022, length at toe-off: MG 0.964±0.013 vs 0.953±0.113, p=0.032, soleus 0.983±0.014 vs 0.967±0.0178, p=0.021), but the range during stance did not differ between the groups. At matched speed, no significant differences were observed between YOUNG and OLDER in MTU lengths or ranges. MG MTU range significantly increased in OLDER when walking speed was increased from preferred to matched (0.051±0.012 vs 0.055±0.012, p=0.043).

TT maximal elongation and strain did not differ between the groups, nor did they change with speed in OLDER. Pooling the data from both groups and both speeds of OLDER resulted in the following mean TT maximal elongation and strain values: MG 27.2±5.3 mm and 6.4±1.2%, soleus 16.3±4.7 mm and 5.9±1.7%.

Muscle fascicle and muscle-tendon unit shortening velocities

Soleus peak shortening velocity during push-off was significantly higher at the faster compared to slower walking speed in OLDER (0.35±0.16 vs 0.29±0.14 reference length/s, p=0.010) and a similar tendency was observed in MG (0.63±0.23 vs 0.57±0.16 reference length/s, p=0.066). Fascicle shortening velocity at peak TT length was significantly lower in OLDER compared to YOUNG at preferred speed (MG 0.11±0.10 vs 0.25±0.23 reference length/s, p=0.048, soleus 0.06±0.12 vs 0.20±0.15 reference length/s, p=0.020) but not at matched speed (MG p=0.545, soleus p=0.208). Soleus fascicle shortening velocity at peak TT length was significantly higher at the faster compared to slower walking speed in OLDER (0.13±0.15 vs

0.06±0.12 reference length/s, p=0.004) but a statistically significant difference was not observed in MG (p=0.094).

Muscle-tendon interaction

In both MG and soleus, muscle fascicle length changes were clearly decoupled from the length changes of the MTU. Contributions of muscle fascicle to MTU length changes during stance were significantly lower in OLDER compared to YOUNG at preferred speed in both muscles (MG 0.26±0.10 vs 0.16±0.05, p=0.012, soleus 0.23±0.08 vs 0.15±0.04, p<0.001, fig 3). Significant differences were not observed when YOUNG and OLDER were compared at matched speeds. Increase in walking speed significantly increased muscle fascicle to MTU length change ratio in OLDER (MG 0.16±0.05 vs 0.19±0.07, p=0.013, soleus 0.15±0.04 vs 0.21±0.08, p=0.002).

Muscle fascicle velocities were clearly different from MTU velocities. Muscle fascicle relative to MTU peak shortening velocities during push-off did not significantly differ between YOUNG and OLDER or within OLDER between different walking speeds in either muscle (fig 3). Pooling both groups and velocities together, MTU peak shortening velocities during push-off were on average 8 and 11 times greater than peak shortening velocity of the muscle fascicles in MG and soleus, respectively.

Electromyography

RMS EMG activity of MG and soleus did not significantly differ between YOUNG and OLDER at either walking speed when compared during stance or push-off. However, EMG activity significantly increased in both stance and push-off in both muscles in OLDER as they increased their walking speed from preferred to matched speed (MG stance 0.32±0.12 vs 0.38±0.16 RMS/RMS_{MVIC}, p=0.011, push-off 0.39±0.17 vs 0.47±0.23 RMS/RMS_{MVIC}, p=0.026,

soleus stance 0.36±0.18 vs 0.40±0.19 RMS/RMS_{MVIC}, p=0.001, push-off 0.43±0.23 vs 0.49±0.23 RMS/RMS_{MVIC}, p=0.001.).

Ground reaction forces

Peak propulsive and resultant forces during push-off significantly increased in OLDER with increase in walking speed (propulsive 0.16±0.03 vs 0.19±0.03 body weights, p<0.001, resultant 1.03±0.07 vs 1.05±0.08 body weights, p=0.012). However, both peak propulsive and resultant forces during push-off remained significantly lower in OLDER compared to YOUNG at matched speed (propulsive 0.19±0.03 vs 0.22±0.04 body weights, p=0.011, resultant 1.05±0.08 vs 1.14±0.08 body weights, p=0.045).

Associations between preferred walking speed and triceps surae muscle-tendon function

A significant positive correlation was observed between preferred walking speed and body weight-normalized plantarflexion strength (r=0.562, p=0.046). Preferred walking speed was also significantly correlated with MG mean fascicle velocity during push-off (r=-0.461, p=0.018), mean soleus fascicle velocity during push-off (r=-0.534, p=0.006), minimum soleus fascicle velocity during push-off (r=-0.599, p=0.002), soleus fascicle velocity at estimated peak force generation (r=-0.653, p<001), soleus fascicle to MTU length change ratio during stance (r=0.412, p=0.041), soleus muscle fascicle operating range during stance (r=0.603, p=0.001) and MG TT peak strain (r=0.399, p=0.043). Linear regression analysis showed that age only had a significant effect (p<0.05) on the above relationships for MG mean fascicle velocity during push-off and soleus fascicle to MTU length change ratio during stance.

Discussion

The current study examined triceps surae muscle-tendon function in young and older men during walking. No significant differences were observed between the young and older men at matched walking speed in any triceps surae muscle-tendon function parameters. Only peak GRFs during push-off were significantly lower in older compared to young men. However, at their preferred walking speeds, older men utilized a narrower fascicle operating range, had lower fascicle shortening velocity at estimated peak force generation, and fascicle length changes accounted for a lower proportion of MTU length changes compared to young men. These findings may partly explain why older adults prefer to walk at a slower speed.

The results of the current study did not support our first hypothesis. We did not observe differences in TT elongation between young and older men when the groups were compared at the matched speed, which we expected based on previous studies that showed lower Achilles tendon stiffness in older compared to young adults (9, 20, 34, 38). However, our finding is consistent with the results of Csapo et al. (9), showing lower stiffness but similar MG fascicle shortening in older compared to young women in isometric contractions with similar force generation relative to maximal. It could be that aponeuroses, the stiffness of which can vary according to level of muscle force (4), serve to preserve TT length changes despite possible differences in free tendon stiffness between the age groups. We also hypothesized that contractile conditions in triceps surae muscles would be less optimal for force generation according to muscle force-velocity properties in older compared to young men at matched walking speed. The results did not support this hypothesis as no significant differences were observed between the age groups. Thus, it can be concluded that the results do not support the idea that differences in TT behavior or triceps surae muscle fascicle length or velocity between young and older men can explain reduced

plantarflexor performance in older men. However, it still remains possible that significantly less energy (normalized to body mass) was stored in TT of older compared to young men, as suggested by lower peak resultant GRF and similar TT elongation, contributing to previously observed reduction in ankle plantarflexion peak power at late push-off in older men during walking (10, 23). The results however supported our hypothesis that force generation ability in older men improves at preferred walking speed, as fascicle shortening velocity at the estimated time of peak force generation was lower in older men at preferred compared to young adult's preferred walking speed, thus improving muscle force generation ability.

The results of the current study differed from previous studies examining triceps surae muscle fascicle behavior in young and older adults in walking (31, 36). Previous studies have shown differences in either fascicle length change pattern or operating length between the age groups when compared at matched walking speeds (31, 36). A possible reason for the discrepancy between the current and previous studies is that in the current study, spatio-temporal gait parameters were matched between the groups at matched walking speed, whereas in the previous studies, older adults adopted greater stride frequency and shorter stride length to walk at the same speed as young adults, which may have an effect on muscle function despite the matched walking speed. It could be that the older men in the current study were in better physical condition compared to the older adults in the previous studies that reported differences in spatio-temporal gait parameters at matched walking speed between young and older adults. This may in turn explain the discrepancy in the findings related to spatio-temporal gait parameters.

The finding that triceps surae muscle-tendon function was not significantly different between young and older men at matched walking speed but differences were observed at preferred speeds suggest that triceps surae muscle-tendon kinematics are mainly determined by walking

speed without significant effects of age. This was supported by the significant correlations observed between preferred walking speed and triceps surae fascicle shortening velocities, soleus operating range and muscle-tendon interaction and MG TT strain. Age had significant effect on these associations only in few variables in which cases the significant age effect was caused by significant correlations within older but not young men. Hence, the associations between walking speed and muscle-tendon function were not different between the age groups or were only evident in older men.

Walking slower than young men's preferred speed improved triceps surae contractile conditions for force generation by reducing shortening velocity at estimated peak force generation. Thus, at preferred walking speed older men can generate the same force with lower activation and hence energy cost compared to faster walking speeds. This is highlighted by the results of the current study showing that an increase in walking speed of 22% in older men was accompanied by 19% and 13% increases in MG and soleus RMS EMG, respectively, during push-off, but only a 2% increase in peak resultant GRF. In addition, at preferred walking speed in older men, triceps surae muscles operated within a narrower range, thus possibly performing less mechanical work, and TT elasticity also accounted for a greater proportion of the MTU length changes. These walking speed-related adaptations in triceps surae muscle-tendon function may provide a mechanism to mitigate the age-related reduction in plantarflexor force generation capacity, and to reduce the energy cost of walking.

We propose that the walking speed-related adaptations in triceps surae muscle-tendon function observed in the current study could play a role in the selection of a slower preferred walking speed in older compared to young adults. In support of the idea that plantarflexor force generation is an important factor in the selection of preferred walking speed in older adults, we

observed a significant correlation between preferred walking speed and body weight-normalized plantarflexion strength. Previously, contractile conditions for force generation in triceps surae muscles have been linked to preferred walk to run transition speed in young adults in both experimental and modeling studies (11, 33), and a similar mechanism could be involved in the selection of preferred walking speed of older adults. Improved contractile conditions for force generation could also play a role in the selection of preferred walking speed via energy cost of walking. Humans tend to prefer walking speeds that minimize gross energy cost of walking per unit distance (6, 12). Triceps surae muscles may make a relatively large contribution to changes in whole body energy cost in walking with changes in walking speed since it has been shown using EMG-driven simulations that triceps surae were the only muscles of the 11 analyzed lower limb muscles that decreased their force generation ability with increasing walking speed, requiring greater activation to maintain required force generation (3). Thus, improvement in triceps surae contractile conditions for force generation and consequential decrease in muscle activation to maintain required level of force generation may affect total energy cost of walking and hence drive the selection of slower walking speeds in older adults. In addition, it has been shown that older adults compensate for reduced plantarflexor power and work output during push-off by increasing the use of hip extensors in early stance (10), which may operate with greater reserve capacity (23). However, this distal to proximal shift in muscular strategy, where power generation of plantarflexors that can utilize a catapult action due to a long Achilles tendon (21) is replaced by power generation from an MTU with a short tendon, may increase energy cost of walking due to unfavorable muscle shortening velocities of the muscles providing the positive power (37). In addition, the distal to proximal shift in muscular strategy in older adults, which is associated with more conserved center of mass trajectory (13), may increase energy cost due to

less efficient pendulum-like mechanical energy exchange (35). Hence, improved contractile conditions for force generation in plantarflexors due to slower walking speed may increase the proportion of plantarflexor power output to the total positive power, which may be a more economical strategy. Finally, it should be noted that walking is a complex motor action and several different muscular strategies can be utilized in walking. It is also probable that other factors than those mentioned above affect selection of preferred walking speed. For example, it has been previously suggested that older adults may purposefully limit propulsion in push-off to improve balance (14). Thus, it is likely that no single factor is solely responsible for selection of preferred walking speed.

Limitations

A limitation of the current study is the two-dimensional nature of ultrasound imaging. Error in the measured fascicles lengths could arise from out of plane motion of the fascicles and from the imaging plane not being completely aligned with the fascicle direction (5). In addition, muscle fascicle lengths were measured from a single location and thus it is not known how well the results reflect muscle fascicle behavior in other parts of the muscles. However, the measurement location that was used in the current study should represent the general pattern of fascicle length changes, at least for MG (25). Furthermore, these limitations may induce errors in the absolute values but probably do not affect between or within group comparisons. Finally, our study was limited by the lack of inverse dynamics based estimations of muscle-tendon unit mechanics and energetics. These measures would provide important additional insights to muscle function.

Suggestions for future research

To gain further insight into age-related differences in triceps surae muscle function in walking, a larger range of speeds should be tested from both young and older adults with inverse dynamics based estimates of joint torques, powers and muscle forces. These measurements, in combination with measurement of respiratory gases, could provide further evidence for whether selection of walking speed depends on factors related to energy cost, muscle force generation or perhaps other factors such as balance, as previously suggested (14). In future it is also important to examine the effects of different exercise interventions on muscle function and walking speed in older adults. Of particular interest could be interventions specifically aimed to strengthen the soleus muscle and to increase TT stiffness. One type of training intervention that could be used for this purpose is high force isometric strength training that has been shown to be effective at increasing Achilles tendon stiffness (2).

Conclusions

Understanding mechanisms underlying the age-related decline in preferred walking speed is important to be able to target preventative and rehabilitative strategies more effectively, and to better understand the link between reduced walking speed and adverse health outcomes. The results of the current study suggest that force generation ability of triceps surae muscles is decreased in older men when increasing walking speed from their preferred walking speed to the preferred walking speed of young men. In addition, this change in walking speed increases the proportion of fascicle to muscle-tendon unit length change, suggesting less effective utilization of tendinous tissue elasticity. The reduction in preferred walking speed with aging may provide a means to compensate for the decline in triceps surae muscle strength and to minimize the energy

cost of walking. Improving plantarflexor strength could be an effective strategy to improve and maintain walking speed in older age.

Acknowledgements

We thank Patricio Pincheira, Annamaria Péter and András Hegyi for assistance with the study. We also thank all subjects for their participation. This study was supported by grants from the Finnish Cultural Foundation and Emil Aaltonen Foundation to L.S.

Conflict of interest

The authors have no financial or other conflicts of interest to declare. The results of the present study do not constitute endorsement by ACSM.

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Figure legends

Figure 1. Group mean normalized muscle fascicle lengths (A, F) and velocities (B, G), muscle-tendon unit (C, H) and tendinous tissue lengths (D, I) and EMG (E, J) envelopes. The left column shows results from medial gastrocnemius (A-E) and the right column from soleus (F-J). Vertical lines represent start of push-off phase and toe-off respectively. Standard deviations are omitted for clarity.

Figure 2. Normalized medial gastrocnemius (A) and soleus (B) fascicle operating lengths during the stance phase. Whiskers represent standard deviations of lower and upper limit. Crosses mark fascicle lengths at peak tendinous tissue elongation. Fascicle lengths are normalized to resting length at 90° ankle angle and knee extended.

Figure 3. Ratio of muscle fascicle to muscle-tendon unit length change during stance (A) and ratio of muscle fascicle to muscle-tendon unit peak shortening velocity during push-off (B).

Figure 1

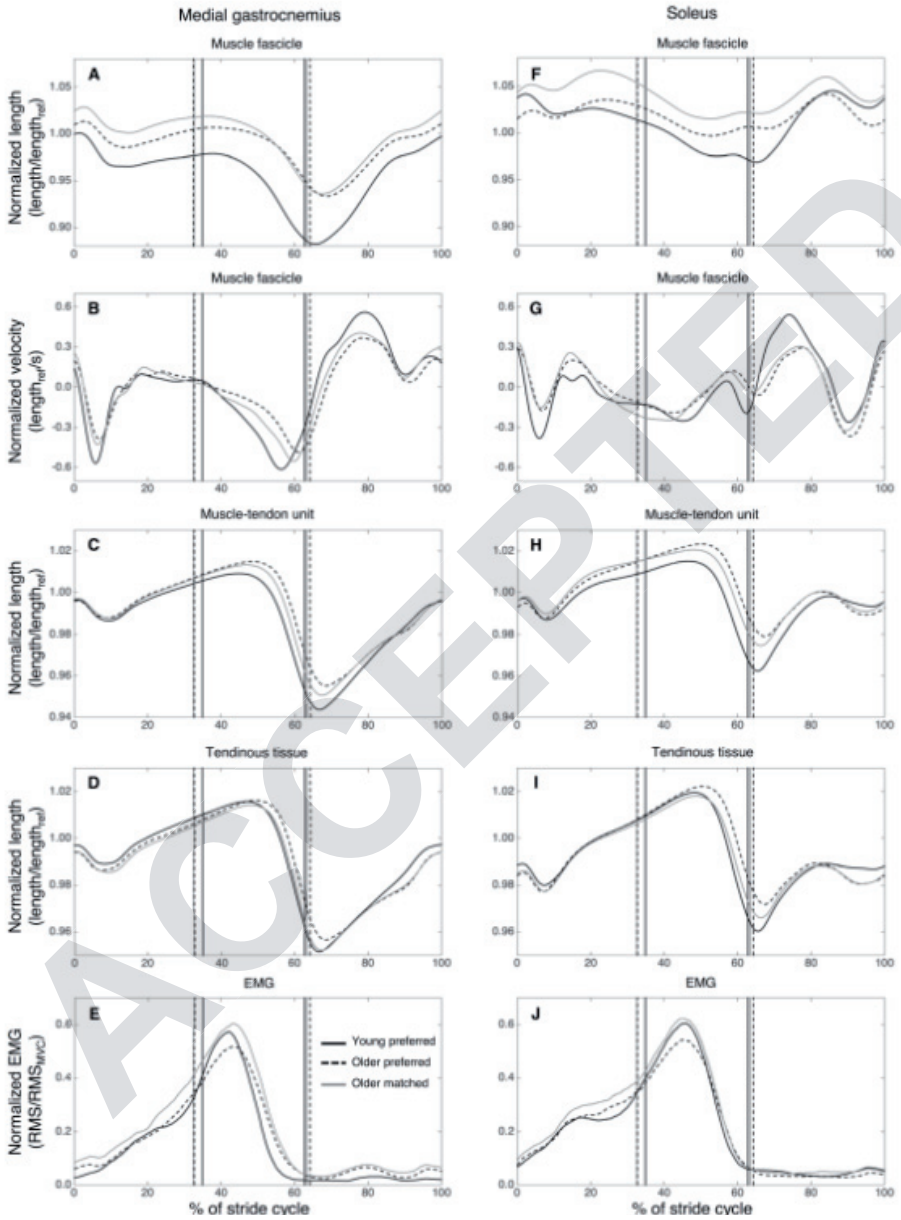

Figure 2

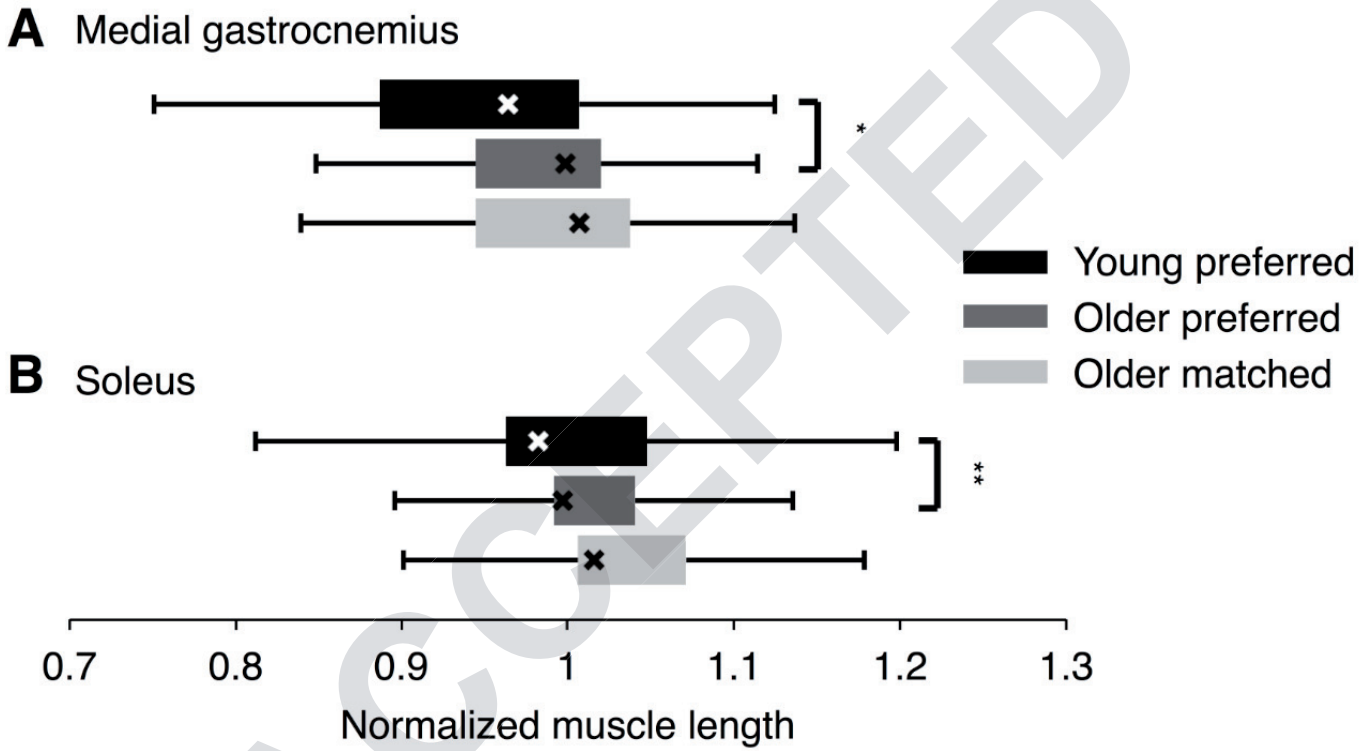

Figure 3

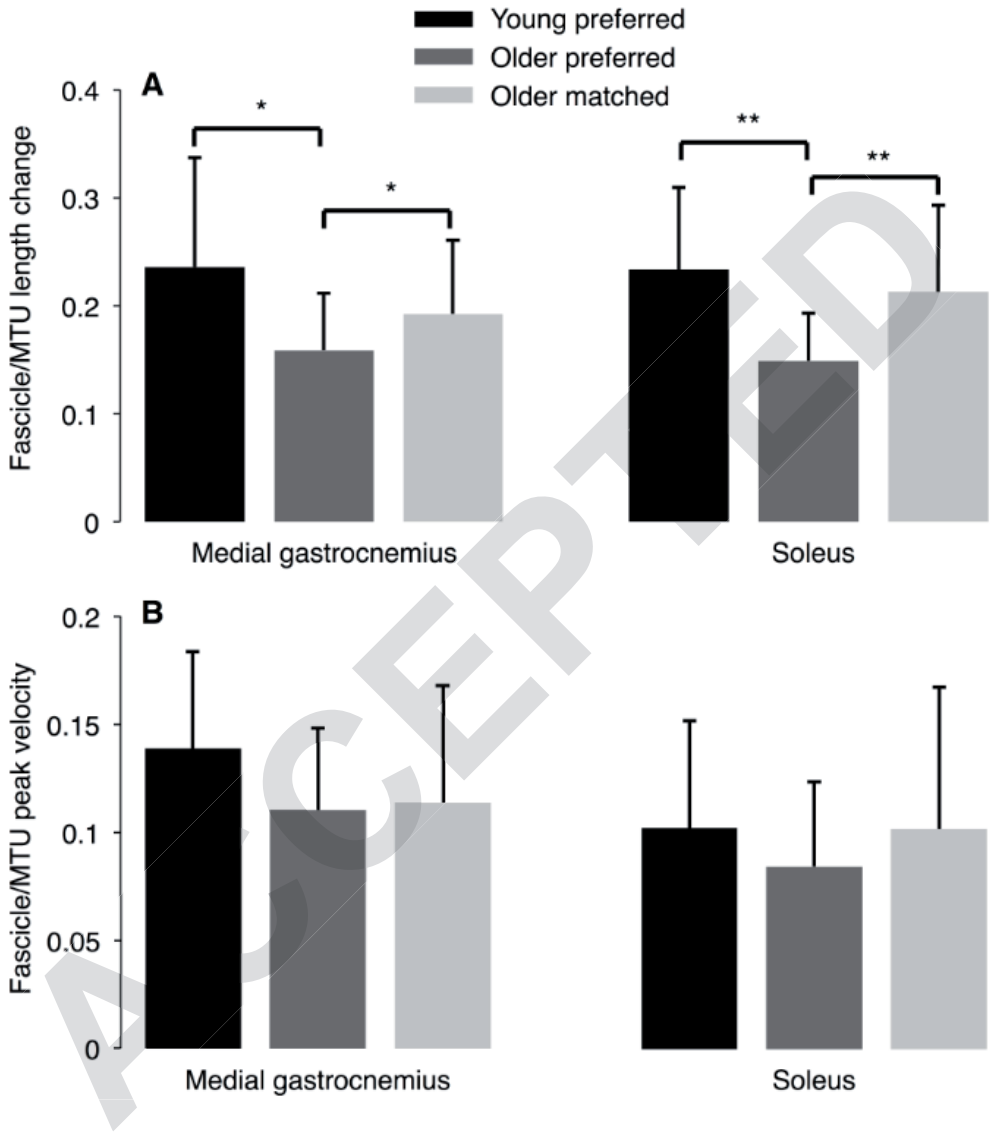

TABLE 1. Subject characteristics

	Young (n=13)	Older (n=13)
Age (yr.)	25 ± 4	73 ± 5
Height (cm)	179 ± 6	176 ± 5
Body mass (kg)	79 ± 7	88 ± 9
BMI (kgm-^{2})	25 ± 2	29 ± 3
Plantarflexion strength (N/body weight)	1.86 ± 0.43	1.30 ± 0.33**
MG fascicle reference length (mm)	52.9 ± 8.6	49.1 ± 5.4
MG MTU reference length (mm)	477 ± 24	469 ± 24
MG TT reference length (mm)	427 ± 18	423 ± 22
Soleus fascicle reference length (mm)	45.0 ± 12.5	41.1 ± 5.0
Soleus MTU reference length (mm)	321 ± 16	316 ± 16
Soleus TT reference length (mm)	280 ±18	281 ± 16

Values are expressed as mean ± SD. BMI body mass index, ** significantly different from young adults ($p<0.01$). Sample descriptors (age, height, body mass and BMI) were not statistically tested for group difference.

TABLE 2. Spatio-temporal gait parameters.

	Young preferred	Older preferred	Older matched
Walking speed (ms^{-1})	1.35 ± 0.16	1.11 ± 0.12**	1.35 ± 0.02##
Stride frequency (Hz)	0.92 ± 0.05	0.85 ± 0.06**	0.93 ± 0.05##
Stride length (m)	1.47 ± 0.14	1.30 ± 0.11**	1.46 ± 0.07##
Duty factor (% stride)	62.8 ± 1.0	64.2 ± 1.8*	63.3 ± 1.6##

Values are expressed as mean ± SD. * significantly different from young adults, # significantly different from older preferred, */# $p<0.05$, **/## $p<0.01$.