STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH
215

Jussi Peltonen

Achilles Tendon Viscoelastic Properties and Mechanical Responses to a Single Bout of Exercise

JYVÄSKYLÄN YLIOPISTO

STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH 215

Jussi Peltonen

Achilles Tendon Viscoelastic Properties and Mechanical Responses to a Single Bout of Exercise

Esitetään Jyväskylän yliopiston liikuntatieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Agora-rakennuksen auditoriossa 2
joulukuun 19. päivänä 2014 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Sport and Health Sciences of the University of Jyväskylä,
in building Agora, auditorium 2, on December 19, 2014 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2014

Achilles Tendon Viscoelastic Properties and Mechanical Responses to a Single Bout of Exercise

STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH 215

Jussi Peltonen

Achilles Tendon Viscoelastic Properties and Mechanical Responses to a Single Bout of Exercise

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2014

Editors
Taija Juutinen
Department of Biology of Physical Activity, University of Jyväskylä
Pekka Olsbo, Timo Hautala
Publishing Unit, University Library of Jyväskylä

URN:ISBN:978-951-39-5969-2
ISBN 978-951-39-5969-2 (PDF)

ISBN 978-951-39-5968-5 (nid.)
ISSN 0356-1070

Jyväskylä University Printing House, Jyväskylä 2014

ABSTRACT

Peltonen, Jussi Tapio
Achilles Tendon Viscoelastic Properties and Mechanical Responses to a Single Bout of Exercise
Jyväskylä: University of Jyväskylä, 2014, 78 p.
(Studies in Sport, Physical Education and Health
ISSN 0356-1070; 215)
ISBN 978-951-39-5968-5 (nid.)
ISBN 978-951-39-5969-2 (PDF)

When excised tendons are cyclically loaded, their maximum tensile strength is reduced. This is known as tendon fatigue and is accompanied by a reduction in stiffness, the gradient of the force-length curve. As tendon fatigue has been linked to tendon damage, it may constitute a mechanism for tendon overuse injuries. The current thesis investigated Achilles tendon (AT) fatigue by measuring its stiffness after two different exercise bouts that were characterized by high-intensity and long-duration. In addition, tendon viscoelastic properties were investigated. As viscous properties arise from internal friction, they are responsible for production of heat and increased temperature of the tissue. This may also damage the tendon. Thus the relation between AT hysteresis and increase of temperature was investigated. AT stiffness and hysteresis were derived from the force-length curves obtained in an ankle dynamometer during voluntary contractions up to 80 % of maximum force. AT force was calculated from measured plantar flexion force and ankle lever arm lengths, and AT length was determined using motion capture assisted ultrasonography. Increase of AT skin temperature was assessed with infrared thermography imaging. The results showed that AT stiffness was unchanged after high-intensity hopping exercise and after a marathon run. Previously, tendon fatigue has been demonstrated in isolated AT's and in vivo after isometric loading in the patella tendon. In hopping and running, AT is unloaded once every movement cycle, and is therefore not exposed to creep stress. This may explain why tendon fatigue was not induced in the current thesis, and why it may be difficult to impose during natural movements. The results of viscoelastic studies showed that AT did not demonstrate strain rate sensitivity within a range of 2–10% s^{-1}. However, AT did demonstrate hysteresis (mean: 9%), and larger hysteresis was related to a larger increase of AT skin temperature during 15 stretch-shortening cycles. Thus, the high variation in hysteresis that is typically observed in vivo could be of physiological origin rather than a measurement artefact. The current results provide support for the idea that some tendons are at risk of elevated temperature, which may damage the tendon. The current thesis increases understanding of the mechanisms of physical activity-related tendon injuries.

Keywords: stiffness, hysteresis, ultrasonography, tendon fatigue, fatigue damage, hyperthermia

Author's address

Jussi Peltonen, MSc
Department of Biology of Physical Activity
P.O. Box 35, 40100
jussi.peltonen@jyu.fi

Supervisors

Professor Janne Avela, PhD
Department of Biology of Physical Activity
University of Jyväskylä

Professor Taija Finni, PhD
Department of Biology of Physical Activity
University of Jyväskylä

Reviewers

Professor Adamantios Arampatzis, PhD
Department of Training and Movement Sciences
Humboldt-Universität Berlin
Germany

Professor Costis Maganaris, PhD
Research Institute for Sport and Exercise Sciences
Liverpool John Moores University
United Kingdom

Opponent

Professor S. Peter Magnusson, PT, DSc
Musculoskeletal Rehabilitation Research Unit &
Institute of Sports Medicine Copenhagen
Bispebjerg Hospital
Faculty of Health & Medical Sciences
University of Copenhagen
Denmark

ACKNOWLEDGEMENTS

The studies for this thesis were carried out at the laboratories of the Department of Biology of Physical Activity in Jyväskylä Finland. These laboratories offer unparalleled facilities and human talent to conduct biomechanical research. Therefore I am thankful to Professor Emeritus Paavo Komi, the head of the department at the time when I began my studies, for accepting me as part of his crew. I would like to express my sincere gratitude to both of my supervisors: Professor Janne Avela, for his endless support throughout the process and Professor Taija Finni, for her teaching and daily inspiration.

I am most indebted to Dr. Neil Cronin, co-author and friend, for making the hours at lab so enjoyable and unselfishly offering his help when required. Without his contribution, the quality of the thesis would have been considerably less. I am happy to thank my international collaborators as well as my numerous fellow Ph.D. students in Finland for their fruitful cooperation. Beside mind bobbling conversations about tendons and life, I am much obliged to Lauri Stenroth, co-author in two papers, for his skilled assistance.

I am grateful to technical staff of the department, particularly Markku Ruuskanen and Sirpa Roivas, for their expertise with all the devices, and to department's secretaries, Katja Pylkkänen and Minna Herpola, for their willingness to help in daily matters.

The financial support from Ellen & Artturi Nyyssönen foundation is also acknowledged.

Finally, I am thankful to my parents, Esko and Leena, for their unconditioned love and support during my journey and to my sister, Noora, for being a trustworthy companion at all times.

Jyväskylä 14.10.2014
Jussi Peltonen

LIST OF PUBLICATIONS

The present thesis is based on the following original papers, which are referred to in the text by their roman numerals:

I Peltonen J, Cronin NJ, Avela J, Finni T (2010). In vivo mechanical response of human Achilles tendon to single bout of hopping exercise. Journal of Experimental Biology. 213 (8): 1259–65.

II Peltonen J, Cronin N, Stenroth L, Finni T, Avela J (2012). Achilles tendon stiffness is unchanged one hour after a marathon. Journal of Experimental Biology 215(20): 3665–71.

III Peltonen J, Cronin NJ, Stenroth L, Finni T, Avela J (2013). Viscoelastic properties of the Achilles tendon in vivo. Springerplus 2: 212.

IV Peltonen J, Cronin NJ, Toivonen J, Finni T, Avela J (2014). Relation between Achilles tendon hysteresis and increase of skin temperature during cyclic stretching and shortening in vivo. Submitted for publication.

ABBREVIATIONS

AT	Achilles tendon
COM	Center of mass
COT	Cost of transport
CS	Coordinate system
CSA	Cross-sectional area
EDL	Extensor digitorum longus
ER	Endurance running
IQR	Interquartile range
IRT	Infrared thermography
MG	Medial gastrocnemius
MRI	Magnetic resonance imaging
MTJ	Myotendinous junction
SD	Standard deviation
SDF	Superficial digital flexor
US	Ultrasonography
UTS	Ultimate tensile strength

CONTENTS

ABSTRACT
ACKNOWLEDGEMENTS
LIST OF PUBLICATIONS
ABBREVIATIONS
CONTENTS

1 INTRODUCTION 11

2 LITERATURE REVIEW 13
2.1 History of elastic tendons 13
2.1.1 A short history of the Achilles tendon 14
2.2 Tendon elasticity 15
2.3 Tendon viscoelasticy 18
2.3.1 Hysteresis 18
2.3.2 Strain rate sensitivity 18
2.3.3 Creep 19
2.4 Advantages of tendon elasticity 19
2.4.1 Saving energy 19
2.4.2 Amplifying power 22
2.4.3 Optimizing fascicle length and velocity 23
2.5 Tendon fatigue 25
2.5.1 Tendon mechanical testing 25
2.5.2 Creep and fatigue ruptures 25
2.5.3 Tendon's stress-in-life 27
2.5.4 Challenges of in vitro testing 29
2.5.5 How can tendon fatigue be assessed in vivo? 29
2.6 Achilles tendon 30
2.6.1 Achilles tendon injuries 30
2.6.2 Absorbed heat and increase of temperature 31
2.6.3 Tendon hyperthermia 32

3 AIMS AND HYPOTHESIS 33

4 MATERIALS AND METHODS 35
4.1 Subjects 35
4.2 Protocol overviews 36
4.2.1 Fatiguing exercises (papers I and II) 37
4.3 Apparatus and collection of data 37
4.3.1 Achilles tendon force 39
4.3.2 Achilles tendon length 40
4.3.3 Treadmill running (paper II) 41
4.3.4 Magnetic resonance imaging (paper II) 41

4.3.5 Infrared thermography (paper IV) 42
4.4 Calculations and statistics 42
4.4.1 Force-length curves 42
4.4.2 Running economy (paper II) 43
4.4.3 Thermal analysis and absorbed heat (paper IV) 45
4.4.4 Statistics 46

5 RESULTS 47
5.1 Exercise-induced tendon fatigue (papers I and II) 48
5.1.1 Exercise duration and intensity 48
5.1.2 Maximum force production 49
5.1.3 Tendon stiffness 49
5.1.4 Validity and repeatability of tendon stiffness 50
5.1.5 Running economy and landing technique 50
5.2 Stiffness vs. force 52
5.3 Tendon viscoelastic properties (papers III and IV) 52
5.3.1 Strain rate sensitivity 52
5.3.2 Hysteresis 53
5.3.3 Hysteresis vs. increase of temperature 54

6 DISCUSSION 56
6.1 Exercise-induced tendon fatigue 56
6.2 Achilles tendon viscoelastic properties 59
6.3 Methodological considerations 61
6.3.1 Known limitations 64

7 CONCLUSIONS 66

YHTEENVETO 68

REFERENCES 71

1 INTRODUCTION

The Achilles tendon (AT) is the strongest tendon in the human body, because it is attached to soleus and gastrocnemius that have large cross-sectional areas (Ward et al. 2009). AT may have to bear forces of 9 kN during sprint running (Komi, Fukashiro & Järvinen 1992). It has been suggested that AT first appeared in our anatomy 2 million years ago and has been crucial to our survival, as it may have assisted in persistence hunting (Bramble & Lieberman 2004). Yet AT is one of the most injured tendons in the human body (Jozsa et al. 1989). As ruptured AT's have shown signs of degeneration (Kannus & Jozsa 1991), the theory of tendon overuse injuries has been developed, which states that if repeatedly overloaded, tendon damage will accumulate and tendon injury occurs (Galloway, Jokl & Dayton 1992).

Excised tendons have been shown to rupture at submaximal loads if they are continuously stressed. At the instant of rupture ultimate tensile strength (UTS), the maximum stress that tendon can bear without rupturing is reduced to half of its original value (Schechtman and Bader, 1994; 2002; Wang and Ker, 1995a; Wang et al., 1995b; Wren et al., 2003). This is known as tendon fatigue, and it has been associated with fatigue damage, as evidenced by disruption of tendon fibers in microscopic images (Fung et al. 2009). Fatigue damage may be the underlying mechanism of tendon overuse injuries. As a reduction in UTS has been shown to be associated with a reduction in Young's modulus (Schechtman and Bader, 1994; 2002; Wang and Ker, 1995a; Wang et al., 1995b; Wren et al., 2003), its derivative, tendon stiffness, can be used as an indicator of tendon fatigue in vivo. However, although tendon fatigue has been previously demonstrated in AT in vitro (Wren et al. 2003), changes in AT stiffness due to a single bout of exercise have never been observed in vivo (Mademli, Arampatzis & Walsh 2006, Farris, Trewartha & McGuigan 2011, Lichtwark, Cresswell & Newsham-West 2013).

AT stores and returns considerable amounts of energy as we walk or run (Alexander & Bennet-Clark 1977). Tendons are remarkably efficient; they return 90–95% of the stored strain energy during elastic recoil. Energy that is not recovered is dissipated as heat and absorbed in the tissue. Dissipation of energy is

due to viscous properties that arise from internal friction caused by tendon length changes. Because tendons have both elastic and viscous properties, they are said to be viscoelastic (Sanjeevi 1982). Absorbed heat raises the temperature of the tendon both in galloping horses (Wilson & Goodship 1994) and in running humans (Farris, Trewartha & McGuigan 2011). This may damage the tendon (Birch, Wilson & Goodship 1997). Hysteresis is defined as dissipated heat divided by stored energy, and is around 7–9% in excised tendons of various species (Bennett et al. 1986). However, in the human AT in vivo, hysteresis values of more than 20% have been reported (Lichtwark & Wilson 2005, Farris, Trewartha & McGuigan 2011, Foure, Nordez & Cornu 2010, Maganaris & Paul 2002, Kubo et al. 2002). This discrepancy has not been explained. Thus it is currently unknown whether in vivo hysteresis of the AT represents a true physiological value or not.

The purpose of the current thesis was 1) to investigate AT fatigue after a single bout of exercise. As AT fatigue has been demonstrated in vitro, but not in vivo, AT was loaded with higher intensity (Kubo et al. 2001a, Mademli, Arampatzis & Walsh 2006) and with a greater number of loading cycles (Farris, Trewartha & McGuigan 2011, Lichtwark, Cresswell & Newsham-West 2013) than in previous in vivo experiments. Tendon fatigue was quantified by measuring changes in AT stiffness before and after the exercise; 2) to investigate AT viscoelastic properties such as hysteresis, strain rate sensitivity, and the relation between hysteresis and increase of AT tendon temperature. Viscoelastic properties, particularly high hysteresis, may be related to tendon heat-induced injuries.

2 LITERATURE REVIEW

2.1 History of elastic tendons

In the 1960's tendons were not thought to be elastic. Early evidence that tendons may behave like springs came in 1964. Cavagna et al. used force plates to measure fluctuations in mechanical energy during running (Cavagna, Saibene & Margaria 1964). They assumed that all changes in mechanical energy are supplied by work done by the muscles. Because they also measured oxygen consumption, they could calculate efficiency of human movement. Efficiency is mechanical work divided by consumed energy. The results showed that efficiency was much higher than 0.25 deduced in bicycle ergometers. The only explanation was that part of the work was done by tendons acting like springs.

Later this was confirmed in hopping kangaroos (Alexander & Vernon 1975). The authors measured ground reaction forces and calculated joint torques, tendon forces and predicted tendon length changes. These findings led to the conclusion that length changes due to the flexion and extension of the joints were mainly due to stretching and recoiling of the tendons, with very little length changes taking place in muscles.

Much more direct evidence came when sonomicrometry crystals were implanted to tendons of wallabies to measure length changes of the muscle fascicles during hopping (Biewener, Konieczynski & Baudinette 1998). Muscle fiber length changes were less than ± 0.5 mm in the plantaris and ± 2.2 mm in the lateral gastrocnemius, representing less than 2% of total fiber length in the plantaris and less than 6% in the lateral gastrocnemius, with respect to resting length. Due to small length changes, little net work was done by either muscle. In contrast, the energy that was stored in the tendons was on average 20 times greater than the work done by the muscles during shortening.

At the same time, sonomicrometry crystals were also implanted to turkeys (Roberts et al. 1997). During running, gastrocnemius muscle fascicles changed length very little during the ground contact phase. Fascicles changed length much more when the foot was off the ground and force production was very

small. Thus it was concluded that most of the work provided by the muscle-tendon-unit during the push-off phase came from elastic recoil of the tendon rather than from concentric contraction of the muscles.

The first human tendon forces were measured by installing a buckle transducer in the AT under local anesthesia (Komi, Fukashiro & Järvinen 1992). The highest AT force, 9 kN, was recorded during running. Perhaps the most remarkable finding was that AT forces were much lower in maximum static jumps (2.2 kN) and countermovement jumps (1.9 kN) than in submaximal hopping (4.0 kN). Due to the force being twice as high, roughly four-times the amount of energy can be stored in the AT in submaximal hopping compared to maximum static or countermovement jumps.

Because sonomicrometry crystals cannot be implanted in humans, information about muscle fascicle behavior became available only through ultrasonography (US). In the first movement study, gastrocnemius medialis muscle fascicles acted almost isometrically at 50 mm during the contact phase of walking, while AT took up most of the length changes (Fukunaga et al. 2001). Isometric action of muscle fascicles was later confirmed during walking, but more interesting results were acquired during running (Lichtwark, Bougoulias & Wilson 2007). Instead of first lengthening and then shortening, gastrocnemius muscle fascicles shortened continuously from 50 mm when the foot hit the ground to 45 mm when foot left the ground. The advantages of minimal changes in muscle fascicle length are discussed in the following sections.

2.1.1 A short history of the Achilles tendon

The Achilles tendon appeared in our anatomy about 2 million years ago and it may have assisted in the pursuit of prey (Bramble & Lieberman 2004). At about that time in Africa, forests started to disappear and large savannas covered the land. Hunting required a new skill, and that may have been endurance running (ER). Humans have no fur and have a lot of sweat glands, and can thus lose heat effectively through evaporation (Carrier et al. 1984). It has been suggested that due to our superior cooling ability, humans are able to outrun even quadrupedal cursors (animals that are specialized for running) in hot conditions (Bramble & Lieberman 2004). Quadrupeds have three gaits, walking, trotting and galloping, whereas humans have two, walking and running. Most quadrupeds, such as dogs, can outrun humans when galloping, but can sustain that speed for only 10–15 minutes in cool conditions (Heglund & Taylor 1988). Quadrupeds' endurance gait is the trot. Human endurance running speed, which often exceeds 4 m s^{-1} and can reach 6.5 m s^{-1} in elite athletes, exceeds the trot-to-gallop transition speed of all other mammals, regardless of size (Lieberman & Bramble 2007). Thus humans are able to outrun most quadrupedal cursors over long distances, especially when it's hot.

If endurance running ability has been important in the evolution of Homo, it must have left markings in the human anatomy. Apes are poor endurance runners, because they live in the trees and their body is adapted for that life. Compared to our closest living relative, the chimpanzee, humans have legs

with many long spring-like tendons and short muscle fascicles that generate force economically. As will be discussed in the following sections, AT is the most important of these tendons. Unfortunately, tracking down the first developed AT is difficult because fascicle and tendon length probably cannot be estimated from the attachment sites of preserved bones. Based on the available findings, however, Bramble and Lieberman have suggested that a developed AT was absent in Australopithecus, and originated around 3 million years ago, probably in the genus Homo (Bramble & Lieberman 2004).

2.2 Tendon elasticity

Elastic structures deform under compressive or stretching forces but return to their initial form when the application of force stops. An example of an elastic structure is a rubber ball. When thrown against a wall, a rubber ball will bounce back due to its elasticity. The same would happen to a spring, if it hit the wall at the right angle. An ideal spring obeys Hooke's law,

$$F = -kx \tag{1}$$

where F is the force that causes the deformation, k is a spring constant and x is the amount of deformation. The minus sign indicates that the force is always a restoring force. Hooke's law is not a fundamental law of physics. It is an experimental law; it has been observed that many materials obey it. When stretched, stored strain energy U of an ideal spring is proportional to the second power of stretch

$$U = kx^2 \tag{2}$$

Human tendons and ligaments are elastic structures. They deform under physiological loading and approximately follow Hooke's law. Therefore they can be compared to springs. An example of tendon in vitro mechanical testing is given in Figure 1A. In vitro refers to a situation where the tendon force-length relation is determined outside of the tendon's living environment. The upward arrow in Figure 1A indicates stretching of the tendon and the downward arrow shortening of the tendon. Thus the ascending and descending limbs of the tendon force-length curve form a loop. The clockwise direction of the loop indicates that some of the stored strain energy is not returned, but is instead dissipated as heat. Since heat is a form of energy, it can be transformed into macroscopic mechanical energy, and vice versa. The ratio between dissipated heat (loop area) and stored strain energy (area under the ascending limb) is called hysteresis. Hysteresis in mammalian tendons is around 5–10% (Ker 1981, Pollock & Shadwick 1994, Bennett et al. 1986). The opposite of in vitro testing, called in vivo testing, is shown in Figure 1B; the tendon is stretched by its own muscle in a natural environment. The gradient of the curve in Figure 1B is equal to the

spring constant and is called stiffness. Its numerical value is 168 N mm^{-1}. Note that the gradient in Figure 1B is almost constant whereas the gradient in Figure 1A is first low and then increases towards higher force levels. A non-linear force-length relation and hysteresis are what separate tendon from an ideal spring. The absence of a low-stiffness region in Figure 1B probably indicates pre-tension at the onset of stretching, either due to muscle activation or due to joint rotation. During in vitro testing, small pre-tension is also often included to bring the tendon towards its linear region.

Thicker tendons have greater stiffness than thinner tendons of equal length. Shorter tendons also have greater stiffness than longer tendons of equal thickness. To compare mechanical properties of different sized tendons, force and elongation are replaced by stress S and strain E, respectively. Now Equation (1) transforms to

$$S = YE \tag{3}$$

where Y is called Young's modulus. Thus Young's modulus is equivalent to tendon stiffness. The difference between Young's modulus and stiffness is that the former depends on the material properties only whereas the latter also depends on the size of the sample. Since stress is defined as force F per cross-sectional area A and strain as change in length ΔL relative to resting length L_0, Equation (3) can be written as

$$\frac{F}{A} = Y\frac{\Delta L}{L_0} \tag{4}$$

The advantage of stress and strain over force and elongation is that material properties of tendons can be compared. This comparison reveals that the gradient of the stress-strain curve is similar in all tendons. Numerically, Young's modulus varies between 0.8 to 1.6 GPa in animal and human tendons (Maganaris & Paul 2002, Wren et al. 2001b, Bennett et al. 1986, Ker 1981).

Tendons store considerable amounts of energy as they are stretched. During running, the Achilles tendon (AT) may store up to 42 joules of energy. At the same time, ankle and knee extensor muscles store from 4.1 to 8.4 joules of elastic energy. Thus a tendon that has only a fraction of the mass of a 1.66 kg muscle may store 10 times more energy (Alexander, 1977). This may be unsurprising, as muscles primarily function is to do work, not to store elastic energy. However, even when the capacity of tendon to store elastic energy is compared to the capacity of muscle to do work, tendons perform remarkably well. Alexander estimated that the energy that tendon can store is 750 J kg^{-1}. This compares favorably to the maximum work that striated muscles can do in a single contraction, 120 J kg^{-1}. Thus six grams of muscle is needed to do the work that 1 gram of tendon can store (Alexander 2003 p. 42).

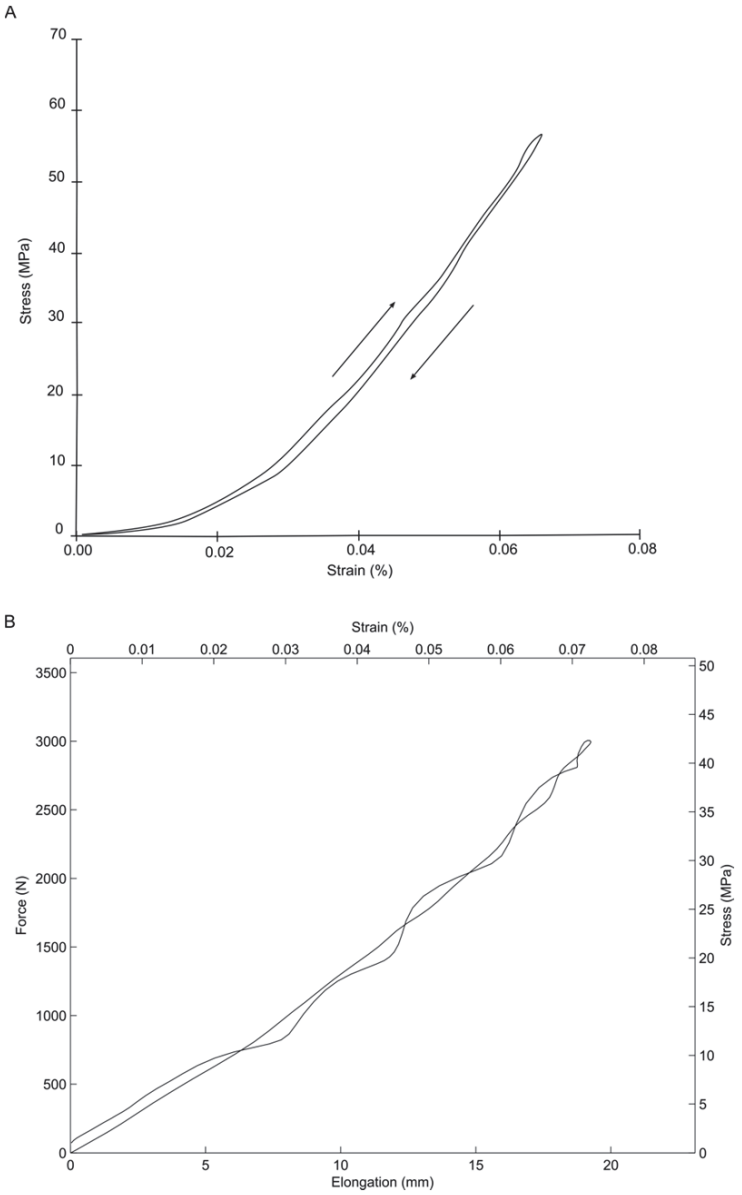

FIGURE 1 Two examples of tendon load-deformation curves. (A) Typical animal stress-strain curve in vitro (Pollock & Shadwick 1994). Arrows indicate direction of the loop (hysteresis = 5.1%). (B) Experimental Achilles tendon stress-strain curve in vivo. Lack of a low stiffness region is due to initial tension acting on the tendon.

Tendons do not store elastic energy endlessly. They break at UTS, the highest stress that tendon can bear without rupturing. The highest known UTS in mammalian tendon is 144 MPa in wallaby tail tendon (Wang & Ker 1995). The

best estimate for UTS in human leg tendons is probably 75–100 MPa (Benedict, Walker & Harris 1968, Wren et al. 2001b, Schechtman & Bader 1997), although this might be an underestimation due to clamping problems often associated with in vitro testing (Ker, Wang & Pike 2000). Due to uncertainty in UTS, it is difficult to obtain reliable estimates of human AT energy at the instant of rupture. Assuming that AT breaks at 80 MPa (Wren et al. 2001b, Wren et al. 2003), the corresponding AT energy is no more than 60 joules. This is only 18% more than AT stores during one-legged hopping (Lichtwark & Wilson 2005).

2.3 Tendon viscoelasticy

Mechanical behavior of viscous materials depends on time. Viscous properties are related to fluids. As biological materials contain water, they typically also demonstrate viscous properties. Tendons demonstrate elastic and viscous behavior and are therefore said to be viscoelastic (Fung 1993, Pioletti et al. 1998, Sanjeevi 1982). Examples of tendon viscoelastic behavior include hysteresis, strain rate dependency and creep.

2.3.1 Hysteresis

Hysteresis is caused by internal friction of a material. This internal friction is responsible for dissipating mechanical strain energy to heat. Hysteresis has been demonstrated consistently in excised tendons from various sources and species, including human AT in vivo. However, there is some controversy in this area. As Figure 19 shows, in vivo hysteresis of the AT is often greater than 20% (Lichtwark & Wilson 2005, Farris, Trewartha & McGuigan 2011, Foure, Nordez & Cornu 2010, Maganaris & Paul 2002). This is in contrast to tendon hysteresis in vitro, which is consistently 5–10% (Pollock & Shadwick 1994, Eliasson et al. 2007, Ker 1981, Riemersma & Schamhardt 1985, Wang, Ker & Alexander 1995, Bennett et al. 1986). Thus it is currently not known how accurately the measured hysteresis in vivo represents tendon physiological hysteresis.

2.3.2 Strain rate sensitivity

The first evidence for strain rate sensitivity came from early failure experiments, which demonstrated that ligaments were more prone to ruptures at high strain rates than at low strain rates (Crowninshield & Pope 1976, Noyes, Delucas & Torvik 1974). As later studies did not find such a relationship (Ng et al. 2004, Danto & Woo 1993, Woo et al. 1990, Wren et al. 2001a, Wren et al. 2001b), it has been postulated that viscoelastic properties may vary between tendons (Wren et al. 2001a). However, stiffness seems resistant to this variation, as evidence suggests that tendon stiffness is independent of strain rate (Abrahams 1967, Ker 1981, Mabuchi, Hayatsu & Fujie 1991, Noyes, Delucas & Torvik 1974, Wang,

Ker & Alexander 1995, Woo et al. 1990, Wren et al. 2001a, Herrick, Kingsbury & Lou 1978).

Fatigue ruptures may also be strain rate dependent. In wallaby tail tendons fatigue ruptures occurred faster than would be predicted from the time to creep rupture (Wang, Ker & Alexander 1995). Fatigue ruptures also occurred sooner at the fast rate (10 Hz) than at the slow rate (1.1 Hz), but there was no difference between intermediate rate (5.3 Hz) and slow or fast rate. These findings may be irrelevant to humans though, as we change stride rate only modestly from 1.5 Hz at the slowest ER speed to 2.0 Hz at the top sprint speed.

2.3.3 Creep

Creep, an increase of length over time at a certain load, does not take place in a tendon unless it is seriously damaged (Wang & Ker 1995). Small (< 1%) creep in the free AT after a 5k run (Lichtwark, Cresswell & Newsham-West 2013) may be related to improper warm up. AT of the medial gastrocnemius (MG) muscle was stretched progressively more during each of the first five stretches after rest, and then reached a constant strain between stretches 6–10 (Maganaris 2003b). Thus creep in the human AT, if it is a physiological property and not an artefact, seems to be related to the first few loading cycles only.

2.4 Advantages of tendon elasticity

Humans advance either by walking or running, as mentioned before. At around 2.3 m s^{-1} we switch from walking to running, because it is more efficient to run (Cavagna & Kaneko 1977). Running can be divided into an ER range, 2.4–6.5 m s^{-1}, and a sprint running range, 6.6–10 m s^{-1}, based on the duration that each can be sustained (Bramble & Lieberman 2004); ER can be sustained for several hours whereas sprint running can be sustained for only a couple of minutes. Note that the highest ER speeds given here equate to elite athletes and individual ER range may vary.

2.4.1 Saving energy

Kinetic and gravitational potential energies fluctuate when we walk or run (Figure 2). This allows energy saving by exchange of energies in both gaits. However, the forms of mechanical energies that are involved in the exchange are different between walking and running. In walking, kinetic and potential energy fluctuate out of phase: kinetic energy reaches its minimum and potential energy its maximum in the mid-stance phase when the supporting leg is relatively straight (Cavagna, Heglund & Taylor 1977). This saves energy as kinetic energy is converted to potential energy and vice versa. In running, kinetic and potential energy fluctuate in phase: both reach their maximum during the flight phase. Thus effective exchange of kinetic and gravitational potential energy is

impossible in running. To solve this problem, elastic strain energy is needed. Elastic strain energy fluctuates out of phase with both kinetic and potential energy during running. When kinetic and potential energies reach their minimum in the mid-stance phase, mechanical energy is still conserved, because it is stored as elastic strain energy (Cavagna, Heglund & Taylor 1977). Thus walking has been compared to an inverted pendulum due to a rigid leg and exchange of kinetic and potential energy. In contrast, running has been compared to a spring-mass-system, because the system is compliant and energy exchange includes elastic strain energy. Differential timing of energy fluctuation has been suggested as the major biomechanical difference between walking and running (McMahon 1985).

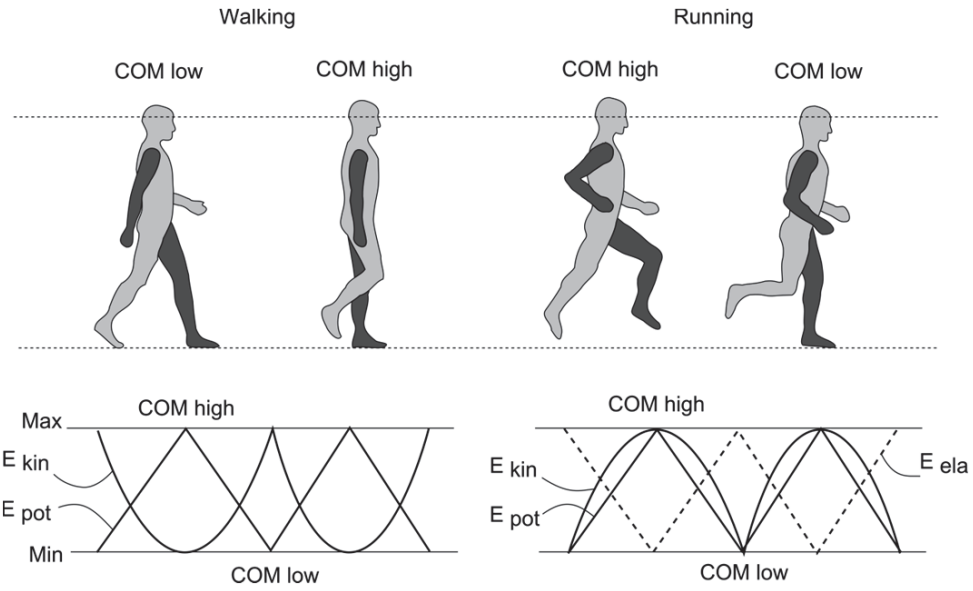

FIGURE 2 Illustration of the exchange of mechanical energy components during walking and running. COM = center of mass; E_{pot} = gravitational potential energy; E_{kin} = kinetic energy; E_{ela} = elastic strain energy. Adapted from Bramble & Lieberman 2004.

Total mechanical energy in running is often determined as the sum of increases in kinetic and potential energy. Alternatively, mechanical energy can also be determined as the sum of internal and external energy, i.e. mechanical energy related to movement of the center of the mass and mechanical energy related to movement about the center of the mass. Either way, total mechanical energy increases with running speed. This is mainly due to an increase in internal energy, i.e. swinging of the arms and legs (Figure 3). At an ER speed of 10 km h^{-1}, total mechanical work is 2 kJ kg^{-1} km^{-1} (Cavagna & Kaneko 1977). To calculate mechanical efficiency of running, net energy expenditure is required. Net energy expenditure is independent of speed, and is around 4 kJ kg^{-1} km^{-1} (Cavagna

& Kaneko 1977). Thus mechanical efficiency at ER speed is 0.5. This is twice as high as could be expected from the efficiency of isolated human muscle (0.23; Smith, Barclay & Loiselle 2005). (Studies of mammalian muscles indicate that efficiency could be even lower, between 0.1–0.2). The only explanation is that some of the increase in mechanical energy comes free of charge. The situation is identical to dropping a rubber ball from a height of 1.0 m and restoring it there. Due to its elasticity, the ball may bounce back to 0.5 m and muscles are required to do only the remaining half of the work. The same applies to running. Although mechanical work increases with running speed, from 2 kJ kg^{-1} km^{-1} at ER speed to 3.2 kJ kg^{-1} km^{-1} at sprint running speed, the net energy expenditure remains the same (Cavagna & Kaneko 1977). As Figure 3 shows, the consequence of this is that the highest mechanical efficiency attainable in sprint running is 0.8. Thus muscles are required to supply only half (0.23/0.5) of the energy during ER and one third (0.23/0.8) of the energy during sprint running than would be required if there were no tendons.

AT is the most important energy storing tendon in the human body. It has been estimated that AT stores 42 joules during running (Alexander & Bennet-Clark 1977). US studies confirm this and add that AT provided 17% of total mechanical energy in hopping (Lichtwark & Wilson 2005). By comparison, the arch of the human foot stores 17 J (Ker et al. 1987). Assuming that the mechanical work of running is supplied by half the muscles and half the tendons (as explained in the previous paragraph), AT and the arch of the foot together may supply 50% of the total elastic strain energy in running.

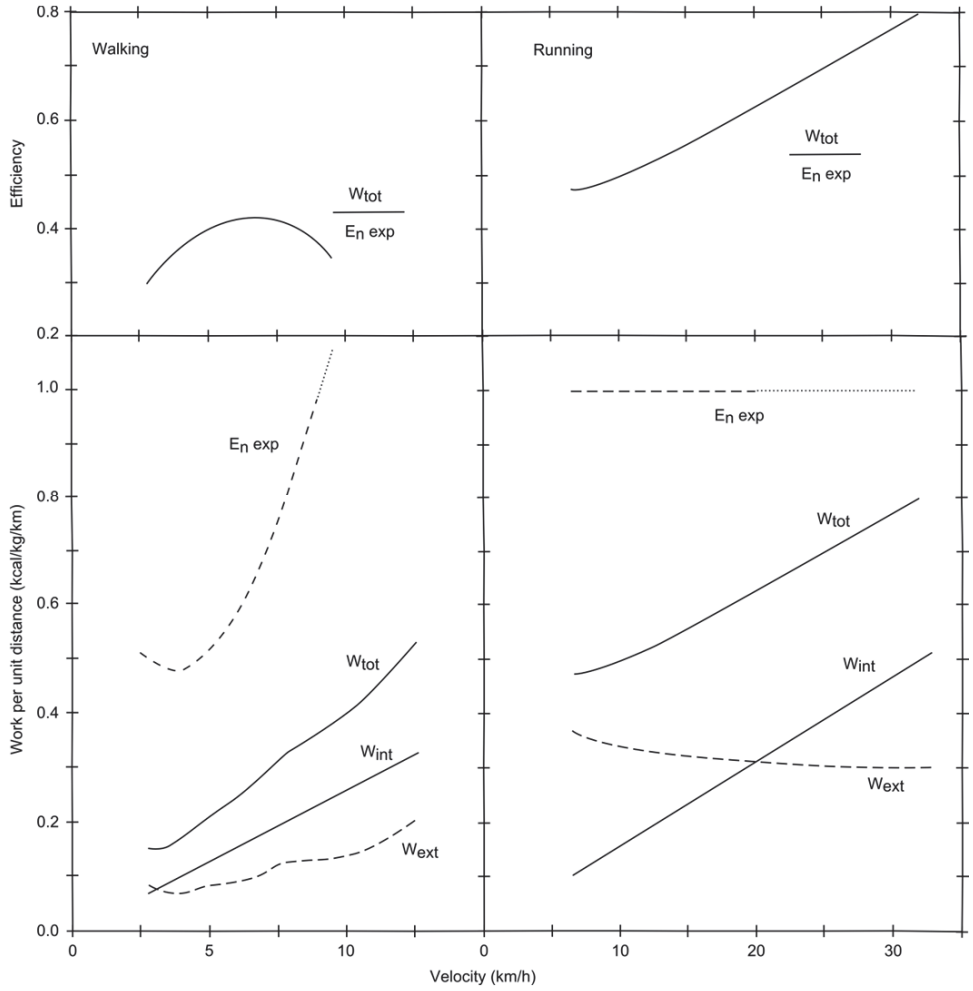

FIGURE 3 Mechanical work, energy expenditure and efficiency of both walking and running at different speeds. **Lower panel** shows internal (W_{int}) and external mechanical work (W_{ext}) and their sum (W_{tot}). Net energy expenditure (E_n exp) in the lower panel is calculated by subtracting energy expenditure in standing from the measured values in walking and running. **Upper panel** shows mechanical efficiency. Adapted from Cavagna & Kaneko 1977.

2.4.2 Amplifying power

Total joint power is calculated by multiplying torque with angular velocity. Ankle joint power is zero in mid-stance of running and reaches a maximum during the second half of the stance phase. Comparing maximum joint power to the maximum power that muscles can supply provides an estimation of how much power elastic structures can transmit. The highest instantaneous power in

mammalian muscle is found in cat soleus muscle (167 W kg^{-1}; Askew & Marsh 1998). To estimate human triceps surae muscle power, the combined mass of soleus and gastrocnemius muscles was taken as 0.45 kg (Ward et al. 2009). Assuming that a sprint runner may have muscles twice that size, the highest power supplied by the ankle extensors is 0.15 kW. This value is 1/19 of the maximum ankle joint power of 2.8 kW in sprint running (Belli, Kyröläinen & Komi 2002). The missing power is due to elastic tendon recoil and transmission of power from upper body segments through the knee to the ankle joint.

To estimate the relative contributions of muscle power, tendon power and transmitted power from upper body segments, ground reaction forces were measured during jumping and a mathematical model was applied to the triceps surae muscle group. This analysis showed that muscle, tendon and transmitted power contributed to 27%, 50% and 20% of the total ankle joint power of 1.8 kW (Bobbert, Huijing & van Ingen Schenau 1986). In absolute terms ankle extensor muscle power was 0.5 kW, three times what would be expected. Using a robotic ankle exoskeleton, another group showed that elastic recoil contributes 60–70% of ankle power when running speed varies between 7 and 12 km h^{-1} (Farris & Sawicki 2012).

2.4.3 Optimizing fascicle length and velocity

Not all muscle lengths are equal. Sarcomeres, which are the building blocks of striated muscles, produce different forces at different lengths. The muscle force-length relation was first studied in frog soleus muscle (Gordon, Huxley & Julian 1966). It was found that sarcomere force is highest at intermediate lengths and the force falls off rapidly when sarcomeres are longer or shorter (Figure 4A). In frogs, optimum sarcomere length was 2.1–2.2 µm. In humans, the best estimate for optimum sarcomere length is higher, 2.64–2.81 µm (for review see Rassier, MacIntosh & Herzog 1999). Thus it would be expected that muscles contract close to their optimum length during movement. This may not always be possible, e.g. when climbing up a tree, during which muscle length changes are large. On level ground, optimum muscle fascicle length can be achieved fairly easily by letting tendons take up most of the length changes in the muscle tendon unit. For example, it has been estimated that MG muscle sarcomere lengths vary between 2.75 and 2.92 µm (Fukunaga et al. 2001) and VL sarcomere lengths between 2.6 and 2.9 µm (Cutts 1989) during most of the stance phase in walking. This saves energy as maximum force is generated by fewer active motor units.

Following the same logic, muscle fascicle contraction velocity should also be adjusted to the optimum value as we walk or run. Fascicles generate the highest force during lengthening, intermediate force when length does not change and the lowest force when length decreases (Edman 1988). As increasing fascicle lengths are almost never observed during natural locomotion, the following discussion focuses solely on fascicle shortening velocities. Figure 4B shows that force declines with increasing shortening velocity and goes to zero at maximum fascicle shortening velocity (v_{max}). Inspection of MG fascicle length with US revealed that fascicles shortened on average at 25 mm s^{-1} during the

stance phase of running (Lichtwark, Bougoulias & Wilson 2007). To compare this to v_{max}, let us assume that v_{max} is four times the fascicle resting length (Edman 1988). If an MG fascicle has a resting length of 50 mm (Ward et al. 2009), its v_{max} is 200 mm s^{-1}. Thus MG fascicles shorten at relatively low velocity, only 0.13 times v_{max}. Farris and Sawicki measured MG fascicle shortening velocities during walking and running and postulated that the reason for switching from walking to running is that it is more economical to run than walk at 2.0 m s^{-1} due to lower fascicle shortening velocity (Farris & Sawicki 2012).

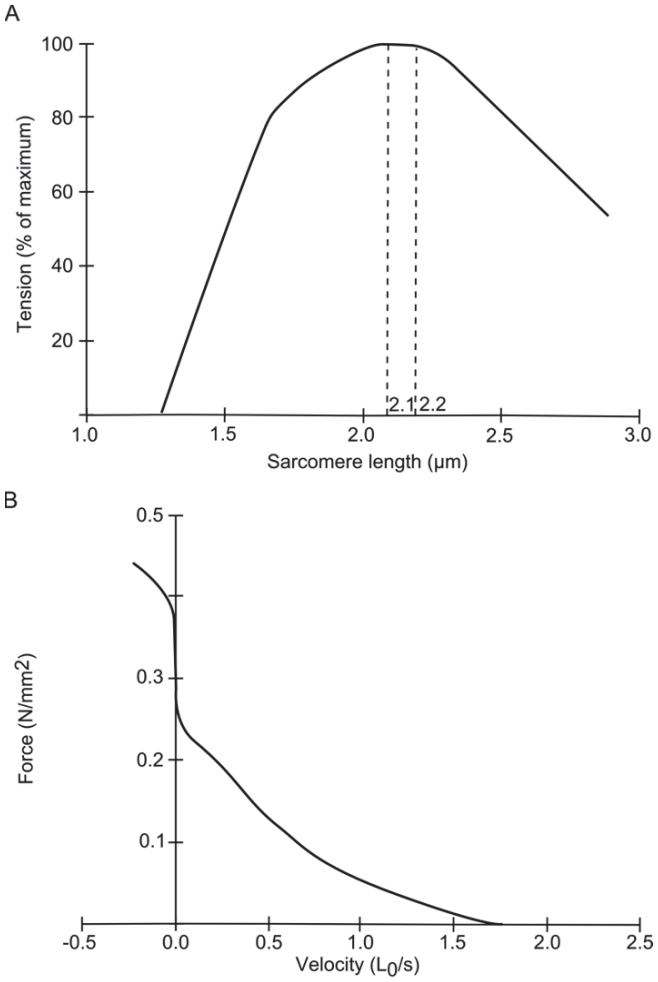

FIGURE 4 Example of muscle (A) force-length (Gordon, Huxley & Julian 1966) and (B) force-velocity relation (Edman 1988).

2.5 Tendon fatigue

It has been suggested that repetitive overloading may damage the tendon and cause tendon overuse injuries (Järvinen et al. 1997). The following sections describe tendon failure testing, induction of creep and fatigue ruptures and how tendon fatigue can be investigated in vivo.

2.5.1 Tendon mechanical testing

A large proportion of tendon mechanical tests, and all failure tests, have been performed with excised tendons. Figure 5 shows results of such a test in the case of human extensor digitorum longus (EDL) tendon (Schechtman & Bader 2002). Initially the tendon does not resist stretch at all or resists very mildly, because tendon fibers are crimped. This is called the low stiffness region or toe region. When tendon fibers are straightened, the tendon starts to resist stretch with a constant stiffness. This region is called the elastic linear region, because the tendon will return to its initial length when application of force stops. Within the elastic linear region, tendon approximately obeys Hooke's law and its behavior may be compared to a spring. It is likely that most natural movements are executed within the elastic linear region. Above the elastic region is the plastic region, where some of the deformation in tendon becomes permanent, as indicated by the imaginary broken line in Figure 5. This means that atoms of the molecules permanently change their positions. However, permanent does not necessarily mean forever, as tendon is a living tissue and is able to repair itself over time. Beyond the plastic region, there is a so called failure region, where tendon is not able to recover from deformation. If application of force continues, the tendon will fail and complete rupture occurs (Wang 2006).

2.5.2 Creep and fatigue ruptures

Both animal and human tendons have been shown to fatigue under continuous static or cyclic loading. In vitro, fatigue is often measured as a decrease in UTS. When rupture occurs after static loading, it is referred to as a creep rupture, and when it occurs after cyclic loading, it is known as a fatigue rupture. Creep or fatigue ruptures have been demonstrated in wallaby tendons (Wang & Ker 1995, Wang, Ker & Alexander 1995, Ker, Wang & Pike 2000) and in sheep tendons (Pike, Ker & Alexander 2000), fatigue ruptures in human EDL tendons (Schechtman & Bader 1994, Schechtman & Bader 1997, Schechtman & Bader 2002), and creep and fatigue ruptures in human AT (Wren et al. 2003). Tendon fatigue has also been associated with tendon damage, as evidenced by disruption of tendon fibers in microscopic images (Fung et al. 2009). To indicate that tendon fatigue is most likely related to tendon damage, some authors have used the term fatigue damage when they have observed changes in UTS of the tendon (Schechtman & Bader 2002). The current thesis uses the term tendon fatigue to indicate a reduction in stiffness of the tendon due to continuous loading.

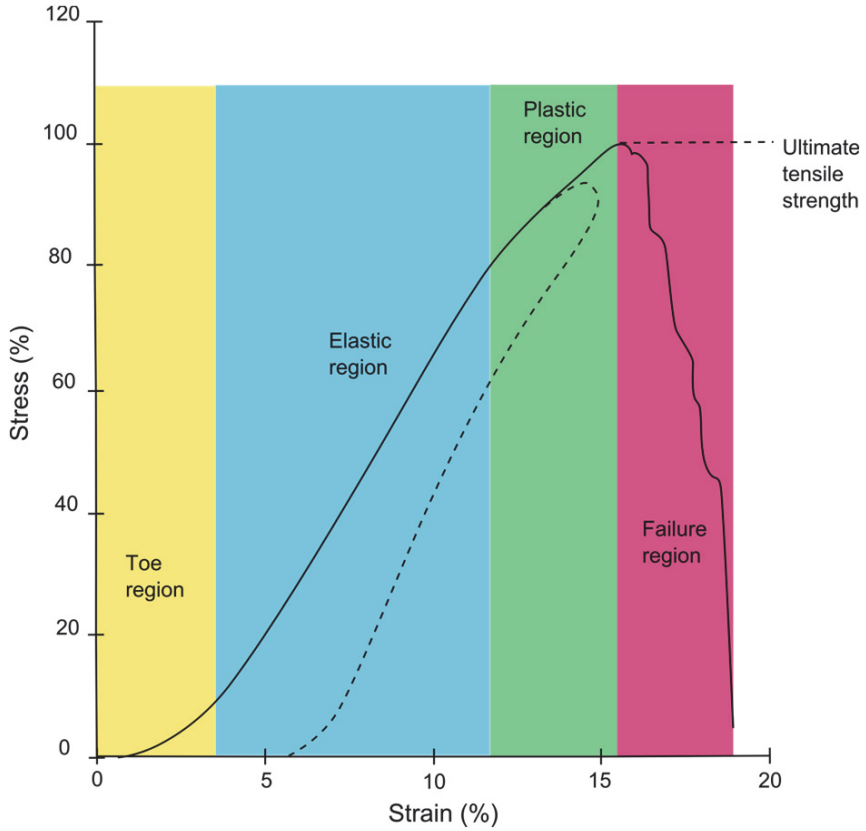

FIGURE 5 Illustration of the tendon stress-strain curve. Data (solid line) are from human extensor digitorum longus tendon (Schechtman & Bader 2002) and the effect of plastic deformation is presented by imaginary data (broken line).

Using data from human EDL tendons, Schechtman and Bader found the relation between normalized peak stress S_{norm} and number of cycles N prior to rupture during a cyclic fatigue test (Schechtman & Bader 1997). That is

$$S_{norm} = 93.98 - 13.13 \times \log(N) \quad (5)$$

where S_{norm} is the peak stress divided by UTS. Assuming a normalized peak stress of 40%, this equation predicts that the number of loading cycles before tendon rupture is 13 000. The same authors repeated the analysis using data provided by Wang and colleagues from wallaby tail tendons (Wang, Ker & Alexander 1995) and developed the following equation (Schechtman & Bader 1997)

$$S_{norm} = 96.5 - 15.8 \times \log(N) \quad (6)$$

This equation now predicts that only 3 767 cycles are required before tendon rupture at the same 40% normalized stress. This equates to less than 1 hour of

running at normal running pace. Pooling all previous fatigue data from wallaby tail tendons (Wang, Ker & Alexander 1995), human EDL tendons (Schechtman & Bader 1997) and human AT (Wren et al. 2003) together (Figure 6), the best estimate for the relation between normalized stress and number of cycles prior to rupture becomes

$$S_{norm} = 95.81 - 13.35 \times \log(N) \quad (7)$$

This indicates that tendon rupture would be expected after 15 000 cycles at a normalized stress level of 40%. When compared to an estimated 20–25 000 strides taken during a marathon run, all participants should be at risk of rupturing their AT before finishing the race. The 40% normalized stress is probably realistic for AT, but is much more than EDL tendon ever experiences (Ker, Alexander & Bennett 1988). The stress that a tendon experiences in life may predict its fatigue resistance, as discussed in the next section.

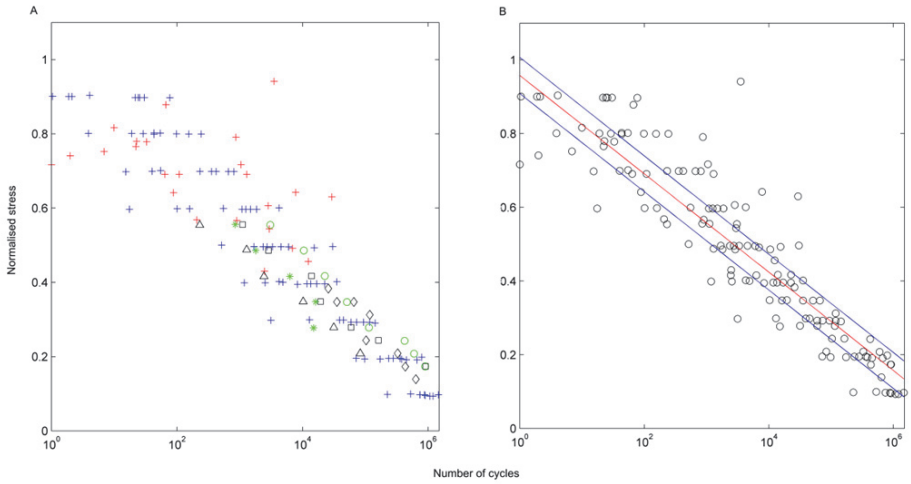

FIGURE 6 Review of the number of cycles required to rupture different tendons. In both figures, the X-axis shows the number of cycles before complete rupture and the Y-axis is the peak stress divided by the ultimate tensile strength of the tendon. (A) Red crosses = Human Achilles tendon at 1 Hz (Wren et al. 2003); blue crosses = human extensor digitorum longus tendon at 2 Hz (Schechtman & Bader 1997); green stars and circles = short (40 mm) wallaby tail tendon at 2.1 and 50 Hz, respectively (Wang, Ker & Alexander 1995); black triangles, squares and diamonds = long (120 mm) wallaby tail tendon at 1.1, 5.3 and 10 Hz, respectively (Wang, Ker & Alexander 1995). (B) Same figure, but without separation by colors, and displayed with the line of best fit (red line) and 50% confidence interval limits (blue lines).

2.5.3 Tendon's stress-in-life

Tendon's stress-in-life is defined as the highest stress that tendon is exposed to in life based on its muscle's cross sectional area (Pike, Ker & Alexander 2000,

Ker, Wang & Pike 2000). To calculate the highest pull that a muscle can exert on its tendon, muscle physiological CSA is multiplied by 0.3 MPa, the maximum isometric stress that muscle can exert (Wells 1965). Even higher stresses can be exerted by muscles that are stretched, but this rarely occurs in natural locomotion: muscle fascicles of turkeys remain mostly isometric (Roberts et al. 1997) and human MG fascicles shorten throughout the stance phase in running (Lichtwark, Bougoulias & Wilson 2007). To yield tendon stress-in-life, the highest pulling force of the muscle is divided by tendon CSA.

Stress-in-life may explain differences in fatigue times between tendons. It was found that tendon's "fatigue quality", time-to-rupture under a given stress, varied hugely among different tendons. When fatigue quality was compared against tendon's stress-in-life, it was shown that the two correlated: tendons that are exposed to higher stress-in-life, based on their muscle's maximum isometric stress, take longer to rupture. This correlation was further demonstrated when tendons from different locations were fatigued at their predicted stress-in-life. Time-to-rupture was almost the same for each tendon, on average 4.2 hours (Ker, Wang & Pike 2000).

Stress-in-life varies in human tendons. EDL tendon's stress-in-life is 12 MPa (Ker, Alexander & Bennett 1988). AT stress-in-life was estimated by taking 82 cm^2 as triceps surae physiological CSA (Ward et al. 2009). Thus AT stress-in-life is given by

$$S_{in-life} = \frac{Muscle\ CSA}{Tendon\ CSA} \times S_{max}iso \tag{8}$$

where S_{max}iso is maximum isometric stress of the muscle, 0.3 MPa (Wells 1965). After substituting muscle physiological CSA with 82 cm^2 and tendon CSA with 70 mm^2 (Wren et al. 2001b), AT stress-in-life is

$$S_{in-life} = \frac{82\text{cm}^2}{0.7cm^2} \times 0.3\text{MPa} = 35\text{MPa}$$

As UTS of the AT and EDL tendons is 80 MPa (Wren et al. 2001b) and 100 MPa (Schechtman & Bader 1997), stress-in-life 39 MPa and 12 MPa (Ker, Alexander & Bennett 1988), their normalized stresses must be 50% and 12%, respectively. The inverse of normalized stress is called tendon safety factor. Using the numbers above, AT has a safety factor of around two and EDL has a safety factor of eight. This indicates that AT and EDL tendons are two and eight times as strong as they are required to be. However, safety factor may not be a good indicator of tendon's susceptibility to ruptures; as long as safety factor is more than one, it is impossible to break a tendon in a single pull, because it is the muscle that will fail first.

Due to its lower safety factor, fatigue rupture would be expected to occur sooner in AT compared to EDL tendon. Studies by Ker et al. suggest the opposite. Tendons with higher stress-in-life have greater time-to-rupture during cyclic or static fatigue tests (Pike, Ker & Alexander 2000, Ker, Wang & Pike 2000). Stress-in-life also increases substantially during maturation in high stressed

tendons, and time-to-rupture increases significantly with this stress (Pike, Ker & Alexander 2000). The same relation is not seen in low stressed tendons. It has been hypothesized that tendons with greater stress-in-life are made up of higher quality material, and are therefore more fatigue resistant than tendons with lower stress-in-life (Ker, Wang & Pike 2000, Pike, Ker & Alexander 2000).

2.5.4 Challenges of in vitro testing

Tendon stress-in-life does not explain why some AT's break earlier than expected during cyclic fatigue experiments. Total AT ruptures have occurred at physiological stress levels of 50 MPa after less than 5000 loading cycles (Wren et al. 2003). It has been postulated that clamping problems may have caused some of the premature failures and underestimations of tendon fatigue resistance in these cases. Tendons that are thick and short are harder to hold compared to tendons that are long and thin (Ker, Wang & Pike 2000). Wren et al. mention that AT is especially hard to work with, and non-uniform strains often occur (Wren et al. 2003). It is possible that clamping problems have resulted in underestimation of UTS too. The measured UTS of the AT, 80 MPa (Wren et al. 2001b), is lower than calculated AT peak stress during sprint running, 110 MPa (Komi, Fukashiro & Järvinen 1992), and slightly higher than during one-legged hopping, 70 MPa (Lichtwark & Wilson 2005).

2.5.5 How can tendon fatigue be assessed in vivo?

Ultimate tensile strength cannot be used to determine tendon fatigue in vivo. Fortunately, most authors have concluded that the slope of the linear region of the stress-strain curve, known as Young's modulus, is an excellent indicator of tendon fatigue. At the instant of rupture, Young's modulus has been shown to decrease to approximately half of its original value (Schechtman & Bader 2002, Wren et al. 2003).

Instead of Young's modulus, tendon stiffness is often used in in vivo studies to quantify tendon fatigue. The difference between stiffness and Young's modulus is that tendon stiffness depends on both material properties and dimensions of the tendon, whereas Young's modulus is determined by material properties alone. In terms of stiffness, if fatigue is induced, tendon stiffness decreases.

A decrease in tendon and aponeurosis stiffness in vivo was first reported in vastus lateralis (VL) muscle after isometric contractions (Kubo et al. 2001b, Kubo et al. 2001a, Kubo, Kanehisa & Fukunaga 2005). By contrast, no changes in stiffness have been observed after dynamic contractions in VL tendon (Kubo et al. 2001a, Kubo, Kanehisa & Fukunaga 2005, Ullrich, Mademli & Arampatzis 2009, Mademli, Arampatzis & Walsh 2008) or in MG tendon (Mademli, Arampatzis & Walsh 2006, Farris, Trewartha & McGuigan 2011, Lichtwark, Cresswell & Newsham-West 2013).

2.6 Achilles tendon

Hierarchical organization of tendons starts from collagen fibrils, which bundle together to form fibers (Figure 7). Collagen fibers form bundles of fibers that are surrounded by a sheet called endotenon. A cross-sectional cut of AT reveals that it is made of bundles of fibers of different sizes. The outer layer of tendon is called epitenon (Kannus & Józsa 1997 p. 50).

Achilles tendon begins as a continuation of the gastrocnemius muscle. On the anterior surface, AT receives fibers from the soleus muscle. The gastrocnemius portion of the tendon ranges from 11 cm to 26 cm and soleus portion from 3 cm to 11 cm. The distal head of the AT attaches to the posterior surface of the calcaneus. AT may spiral up to 90 degrees as it descends, so that posterior fibers become lateral, lateral fibers become anterior, anterior fibers become medial and medial fibers become posterior. The significance of the rotation is that regions of concentrated stress may be produced where fibers from soleus and gastrocnemius meet (Kannus & Józsa 1997).

Tendons consist of 65% to 75% collagen. Collagen fibers run along the length of the tendon and are the smallest sub-unit of a tendon that can be mechanically tested. Longitudinal orientation of the fibers gives tendon great resistance against longitudinal forces, but some fibers are also arranged transversely. Fibers show a spiral formation, which gives tendon its crimped or wavy appearance in microscopic images. Straightening of the crimped fibers is probably responsible for the AT toe region seen in Figure 5 (Kannus & Józsa 1997).

2.6.1 Achilles tendon injuries

It is possible that we have inherited our long and compliant AT from ancestors that used it to assist with hunting. If this is correct, it is interesting to ask why AT is so prone to injuries if it has been so crucial to our survival (Malvankar & Khan 2011).

AT injuries are the nemesis of all athletes. One out of two endurance runners experience AT injury (Kujala, Sarna & Kaprio 2005). AT is the most ruptured tendon in the human body, accounting for 40% of all tendon ruptures. The incidence rate of AT ruptures is 2–8/100 000 per year. It has been postulated that an increase in recreational sports activities were behind the dramatic increase in AT rupture incidence rate experienced in the mid-1980s (Leppilahti & Orava 1998).

A typical AT rupture patient is a male in his forties. The ratio between males and females is on average 6:1 and varies between 2:1 and 19:1. Rupture risk is highest between years 30 and 40, and the mean age of AT rupture patients is slightly higher than in any other tendon. AT rupture is typically unilateral with a slight bias to the left side (Leppilahti & Orava 1998).

Seventy five percent of AT ruptures are associated with sports. The risk is highest in recreational sports that involve explosive movements like sprints,

stops and jumps. Different ball games are involved in 60% of cases, but the most dangerous sport is difficult to name due to national variation. Previous injury or problem is associated with 10% of patients. The most common rupture site, with 80% incidence, is 2–6 cm proximal to the AT insertion on the calcaneus, and it has been proposed that the restricted blood supply in this area may be a predisposing factor (Leppilahti & Orava 1998).

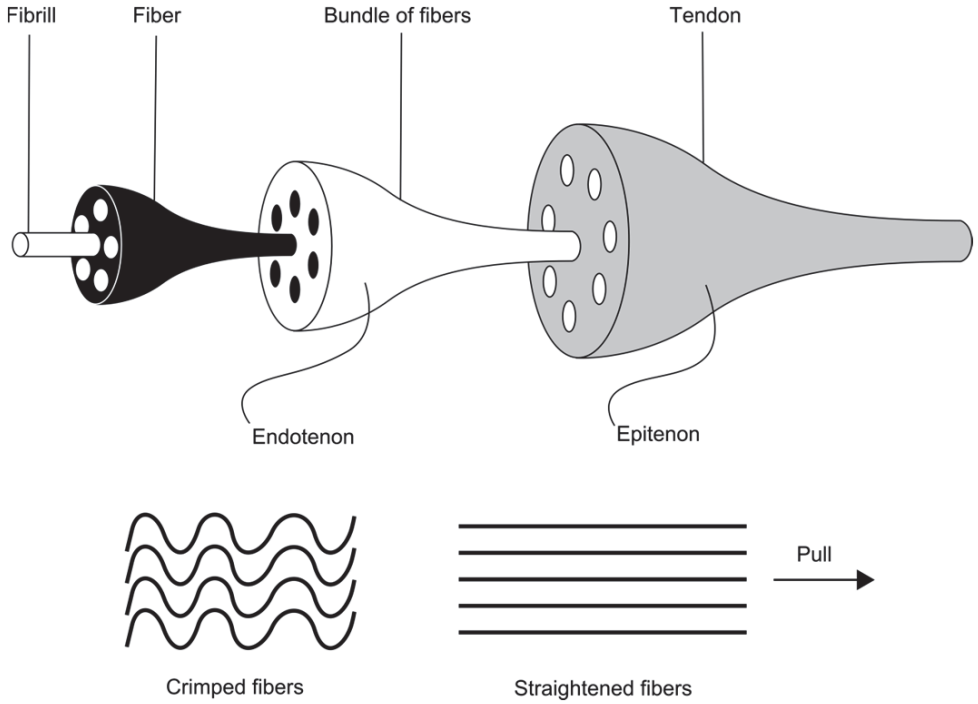

FIGURE 7 Simplified presentation of hierarchical structure of the Achilles tendon. Adapted from Kannus & Józsa 1997.

The mechanisms of tendon injuries are poorly understood. In a study of 891 AT rupture patients, it was found that 97% showed signs of degeneration (Kannus & Jozsa 1991). Thus it has been suggested that mechanical overload during physical activities may damage the tendon and cause tendon overuse injuries (Järvinen et al. 1997).

2.6.2 Absorbed heat and increase of temperature

The Achilles tendon dissipates mechanical energy as heat due to hysteresis. This raises the temperature of the tendon. Absorbed heat ΔQ and increase of temperature ΔT are related,

$$\Delta Q = mc\Delta T \tag{9}$$

where *m* is mass and *c* is specific heat capacity. For example, specific heat capacity of water is 1 kcal kg^{-1} $°C^{-1}$. Thus if the increase of temperature of tendon is known, the corresponding absorbed heat can be calculated, and vice versa.

Increase of AT temperature could be assessed with infrared thermography (IRT). Thermography cameras detect radiation in the infrared range of the electromagnetic spectrum (roughly 9–14 µm). Because infrared radiation is emitted by all objects above absolute zero, thermography makes it possible to see how an object's temperature changes over time. Infrared thermography has previously been used in a wide range of applications from the study of thermoregulation of running dogs (Vainionpää et al. 2012) to clinical diagnostics in humans (Arora et al. 2008). Due to the proximity of the AT to the overlying skin, infrared thermography could potentially also be used to assess AT temperature changes.

2.6.3 Tendon hyperthermia

Hyperthermia is defined as elevated temperature when the body absorbs more heat than it dissipates. Wilson and Goodship measured core temperatures in horse superficial digital flexor (SDF) tendon of 43–45°C after galloping. Using a mathematic model, they showed that AT may reach similar temperatures during vigorous exercise (Wilson & Goodship 1994). A study of cell survival rates of SDF tendon fibroblasts showed that galloping temperatures are not sustained long enough to cause cell death in the tendon central core in vivo. However, it was concluded that repeated hyperthermic insults may compromise cell metabolism of matrix components, resulting in tendon central core degeneration (Birch, Wilson & Goodship 1997). The relation between horse and human tendon core temperatures is not known, but a similar galloping stress of 40–50 MPa (Biewener 1998) compared to AT running stress (Komi, Fukashiro & Järvinen 1992) indicates that temperatures may also be equal. In comparison, galloping is sustained only for 10–15 minutes, whereas human endurance running may continue for several hours. Thus humans could be at higher risk of developing tendon hyperthermia due to their unrivaled endurance running ability.

After 30 minutes of running, AT core temperature was estimated to reach 41°C (Farris, Trewartha & McGuigan 2011). This was based on the assumption that AT hysteresis is 17%. Pooled data of 37 human AT's demonstrate that hysteresis could be even higher, on average 24% (Lichtwark & Wilson 2005, Farris, Trewartha & McGuigan 2011, Foure, Nordez & Cornu 2010, Maganaris & Paul 2002), compared to 5–10% hysteresis in tendon specimens (Ker 1981, Pollock & Shadwick 1994, Bennett et al. 1986). If determined values for AT hysteresis in vivo are correct, it may be postulated that AT is at risk of developing tendon hyperthermia and tendon core degeneration during strenuous exercise due to its high hysteresis, and that risk may be greater in some individuals than others.

3 AIMS AND HYPOTHESIS

In the literature, the effects of dynamic exercise on tendon properties have only been examined after isolated isometric, eccentric and concentric contractions (Kubo et al. 2001a, Kubo, Kanehisa & Fukunaga 2005, Kubo et al. 2001b, Ullrich, Mademli & Arampatzis 2009, Mademli, Arampatzis & Walsh 2006, Mademli & Arampatzis 2008) or after relatively short duration 30 min or 5 km running (Farris, Trewartha & McGuigan 2011, Lichtwark, Cresswell & Newsham-West 2013). Thus, there is a need for a study utilizing a large number of impacts at high strain rates that occur during natural locomotion in sports and physical activities. It was postulated that these goals would be achieved by two different exercises, which were: 1) Two-legged hopping exercise that demonstrates higher loading frequencies (and presumably higher strain rates) than any previous study, where loading frequencies have varied from 0.25 Hz to 1.5 Hz (Kubo et al. 2001b, Mademli, Arampatzis & Walsh 2006, Farris, Trewartha & McGuigan 2011, Lichtwark, Cresswell & Newsham-West 2013); and 2) A Marathon run that induces a greater number of loading cycles than approximately 2700 during 30 min or 5 km running (Lichtwark, Cresswell & Newsham-West 2013, Farris, Trewartha & McGuigan 2011). Based on specimen data, tendon fatigue may be induced after 15 000 loading cycles (Schechtman and Bader, 1994; 2002; Wang and Ker, 1995a; Wang et al., 1995b; Wren et al., 2003) or possibly faster at high loading frequencies (Wang, Ker & Alexander 1995).

Previous studies have shown that hysteresis is a viscoelastic property of mammalian tendons, although it should be low in order to save energy and protect the tendon against heat damage (Alexander 2002). High hysteresis may pose a risk of tendon hyperthermia and subsequent core degeneration during vigorous exercise (Birch, Wilson & Goodship 1997). Hysteresis of the human AT in vivo is reportedly more than 20% (Kubo et al. 2002, Maganaris 2003a, Lichtwark & Wilson 2005) and thus much greater than 7% in vitro (Ker 1981, Pollock & Shadwick 1994, Bennett et al. 1986), and also exhibits high inter-individual variation not seen in excised tendons.

The main aims and hypotheses of the current thesis were:

1. To measure human AT fatigue after a single bout of high-intensity (two-legged hopping) and long-duration (marathon run) exercise. Tendon stiffness was chosen as an indicator of tendon fatigue since its derivative, Young's modulus, has been shown to be a valid indicator of tendon fatigue in specimen studies (Schechtman and Bader, 1994; 2002; Wang and Ker, 1995a; Wang et al., 1995b; Wren et al., 2003). It was hypothesised that if AT is fatigued, stiffness is reduced.
2. To quantify the effects of possible AT fatigue on running economy and running technique.
3. To obtain information about human AT viscoelastic properties such as hysteresis and strain rate sensitivity. It was hypothesised that if hysteresis values of the human AT in vivo are physiologically realistic, they should be close to the values obtain in vitro (< 10%), yet inter individual variation may still exist (standard deviation, SD = 10%), as seen in previous in vivo studies (Kubo et al. 2002, Maganaris 2003a, Lichtwark & Wilson 2005).
4. To investigate the relation between individual variation in AT hysteresis and increase of AT skin temperature. It was postulated that if variation in hysteresis is of physiological origin, greater hysteresis would be related to a greater increase of AT skin temperature.

4 MATERIALS AND METHODS

The current thesis comprises of four studies that each resulted in a scientific publication. Figure 8 outlines the papers, their main purpose and experimental design. Papers I and II investigated exercise-induced AT fatigue by measuring changes in AT stiffness before and after fatiguing exercise. Papers III and IV investigated AT viscoelastic properties such as hysteresis. I have been involved in designing the experiments, collecting the data, interpreting the results and writing the manuscripts in all four papers. In addition to that, I have done all of the data analysis.

4.1 Subjects

Ten subjects participated in study I (TABLE 1). Studies II and III included 12 of the same subjects, and an additional 2 subjects were recruited for study III. Both genders were included, except in study IV, which consisted of only males. All participants were physically active, but none of them were competitive athletes. No single sport background could be identified amongst the subjects. Twelve subjects in studies II and III had been training for long distance running prior to the study, as the study included participation in a marathon run. Participants were informed about the procedures, benefits and possible risks involved in the study, and they all signed a written consent prior to the study. All methods were approved by the local ethical committee and the study conformed to the standards set by the Declaration of Helsinki.

Twelve marathon runners participated in study II. Eight completed a full marathon (42 km) and four completed a half marathon (21 km). Half marathon runners were considered to be less experienced because their target speed over a shorter distance (mean: 10.8 km h^{-1}) was the same as for full marathon runners (mean: 10.9 km h^{-1}). All subjects were physically active with a life-long training background.

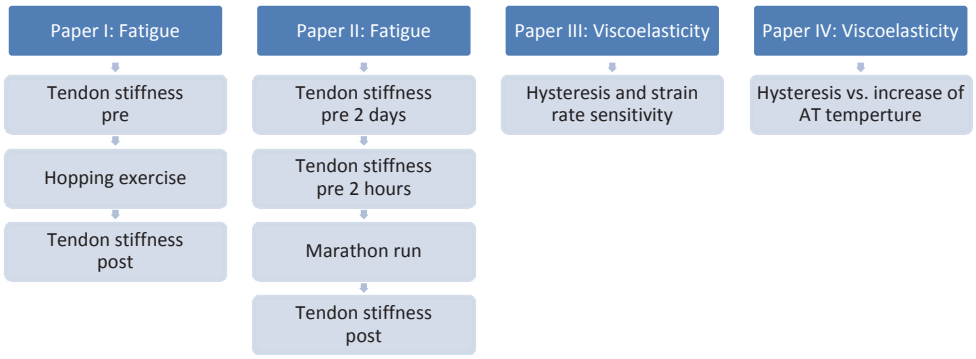

FIGURE 8 Illustration of the experimental design of the four papers including main purpose and experimental session(s).

TABLE 1 Subject gender and mean mass, height and age (± standard deviation) in different studies. N = number of participants, M = male, F = female.

	N (M/F)	Mass (kg)	Height (cm)	Age (y)
1	10 (6/4)	65±10	171±8	26±3
2	12 (8/4)	66±11	172±10	37±13
3	14 (10/4)	67±11	173±11	36±13
4	19 (19/0)	78±12	181±6	27±5

4.2 Protocol overviews

Paper I. AT stiffness was determined before and immediately after fatiguing hopping exercise. Without preparation, study 1 data collection lasted on average 45 minutes.

Paper II. AT stiffness was determined twice before and once after a marathon run. AT stiffness was first tested 2–4 days before the race (pre 2d session) and then again 2-4 hours before the race (pre 2h) to assess test-retest repeatability. Post measurements were conducted 1 hour after the race (post 1h). To examine whether possible changes in stiffness were linked to changes in running economy, ankle kinematics and oxygen consumption were measured during 4 minutes sub-maximal treadmill running in the pre 2d and post 1h tests.

Paper III. AT viscoelastic properties were deduced by measuring AT mechanical behavior at slow and fast strain rates during a single testing session lasting on average 25 minutes without preparation.

Paper IV. To determine the relation between AT hysteresis and increase of temperature, AT mechanical testing was conducted in a single session. AT hys-

teresis was calculated from tendon force-length data, and AT skin temperature was assessed with IRT imaging. Collection of data was conducted in 30 minutes, not including preparation.

4.2.1 Fatiguing exercises (papers I and II)

As 1000 loading cycles at a physiological stress level of 50 MPa has been shown to rupture the AT (Wren et al. 2003) and fatigue ruptures were shown to occur faster at high loading frequencies (Wang, Ker & Alexander 1995), high-frequency hopping exercise was chosen as the loading model for paper I. The exercise consisted of two-legged hopping (feet together) on a contact mat (custom-made, capacitor switch) until exhaustion. Subjects were instructed to hop at their preferred frequency (range: 2.0-2.5 Hz) and strongly activate their calf muscles to keep the heels off the ground during the contact phase. During exercise, flight time ranged from 0.2 to 0.4 seconds (coinciding with a jump height of 5-20 cm) and the total number of jumps ranged between 1150 and 2600.

Based on the cycles-to-rupture vs normalized stress relation [Equations (5–7)], it is predicted that AT ruptures may occur after 3 800–15 000 loading cycles at a normalized stress of 40%. This should be exceeded in running, because 40% normalized stress corresponds to 32 MPa absolute stress, if 80 MPa is taken as the UTS of the AT (Wren et al. 2001b). Using 70 mm^{-2} as AT CSA (Wren et al. 2003), 32 MPa equates to a tensile force of 2240 N. Similar forces act upon the AT already during squat jumps (Fukashiro et al. 1995). Thus a normalized stress of 40% should be exceeded in the AT during running. As a full marathon race consists of approximately 20 000 loading cycles per tendon, runners should be at risk of rupturing their AT even before finishing the race. Thus marathon running was selected as the loading model in paper II. The major differences between papers I and II are that the total number of cycles was greater in paper II but loading frequency was higher in paper I.

4.3 Apparatus and collection of data

Tendon mechanical testing was performed in a custom-built ankle ergometer (University of Jyväskylä, Finland; Figure 10A) that operated in dynamometer mode, i.e. the foot pedal was held stationary. Subjects were seated in a dynamometer chair and their right leg was tightly placed between the foot pedal and a backrest. Their knee was fully extended, ankle at a right angle (sole of the foot perpendicular to the shank) and hip flexed to 60^{o} (0^{o} equals full extension). A force transducer (Precision TB5-C1, Raute, Nastola, Finland) was installed inside the pedal to record foot reaction force. Although the system is non-compliant, the ankle sometimes rotates 1–2 degrees during a maximum push against the pedal. To quantify this small movement, a position-sensitive potentiometer was placed under the heel. This is important for accurate AT length calculation. Pedal force and heel position data were collected with a 16-bit AD-

board (CED 1401, Cambridge Electronic Design, England) at 1 kHz and stored on computer for later analysis.

Achilles tendon mechanical testing started with a warm up of 5–10 submaximal contractions of the calf muscles. Maximal voluntary contraction (MVC) force was then determined as the best out of three trials. Together warm up and MVC trials served as conditioning contractions to stabilize the AT mechanical behavior (Maganaris 2003b). To measure AT force and length data, subjects contracted their calf muscles at a predefined rate and force. Typically the target force was 80 % of MVC. During contractions, the fascicles shortened and AT lengthened the same amount due to a constant ankle angle. At high force levels, small ankle rotations may have occurred, but these were taken into account in the analysis. Force during each contraction was sufficient to extend the AT to the linear region of the force-elongation curve, and thus AT stiffness was reliably determined in all sessions (example in Figure 13).

Different types of contractions used to determine AT mechanical properties are explained in TABLE 2. The difference between ballistic and ramp contraction is in the shape of the force curve. The force trace of ballistic contractions resembles the trajectory of a projectile, whereas force traces of ramp contractions are like a projection of a pyramid. An example of ballistic contractions is shown in Figure 9. Data are from a single subject from study 4 and a total of fifteen contractions divided into three sets of five. AT mechanical properties can be determined from fewer contractions, but as the purpose of study IV was to determine increase of AT temperature, extra contractions were added to generate more heat.

TABLE 2 Description of contraction, its target force and contraction duration during AT mechanical testing in different papers.

Paper	Contraction description	Target force	Duration
I	Slow ramp up, then hold	20–100% of MVC	3–8 s
II	Fast ballistic (↑↓)	80%	1
III	Fast ballistic (↑↓) / slow ramp	80%	1 / 7
IV	Fast ballistic (↑↓)	80%	1

↑ ↓ = Rate of change of force was the same during ascending and descending phases

Achilles tendon stiffness was measured 5–10 min after the hopping exercise bout, but 1–2 h after the marathon. The delay was probably not enough to affect the results, since it has been shown that after mechanical properties reached steady state, they did not change during the following 240 minute recovery period in resting connective tissue (Viidik 1973).

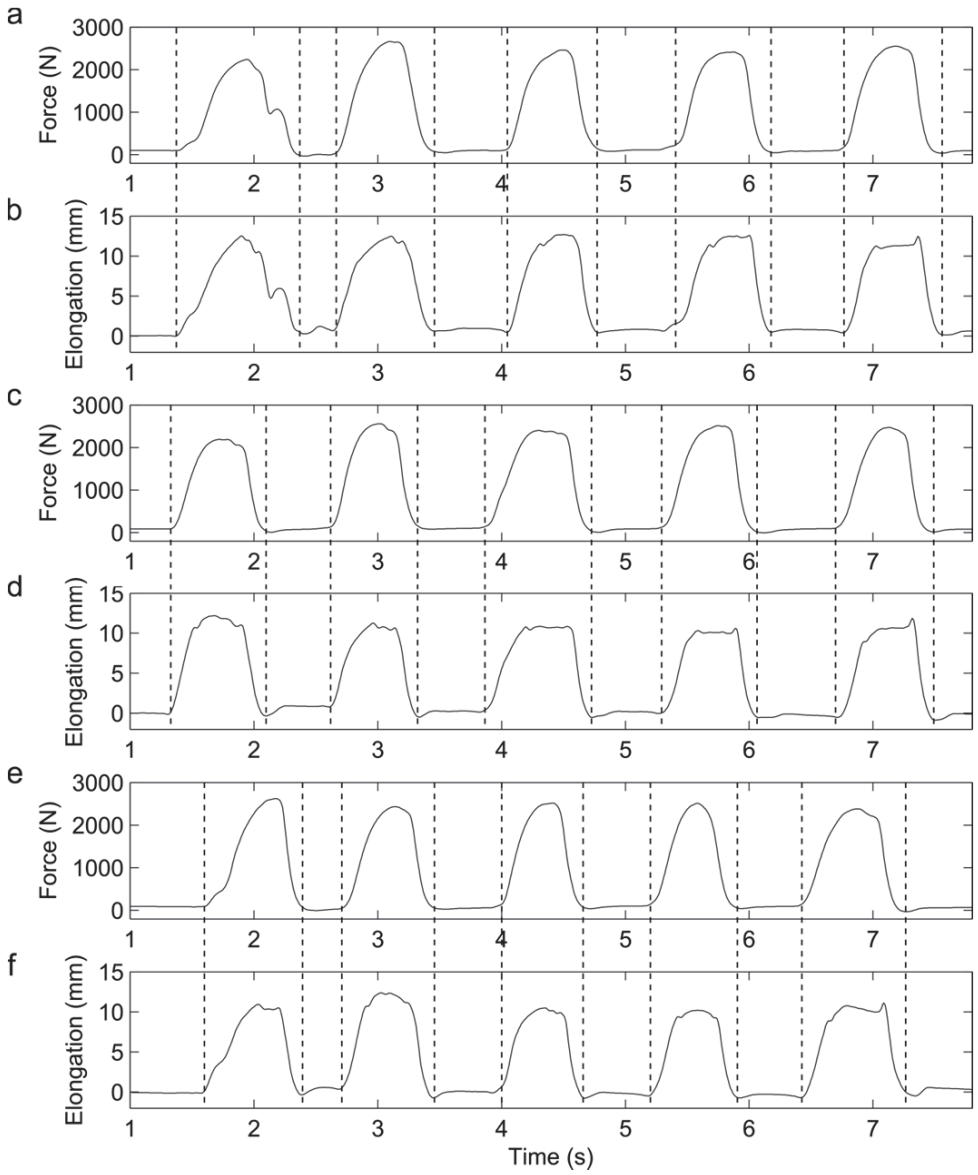

FIGURE 9 Example of calculated Achilles tendon force and elongation data from one subject in study 4. A total of 15 contractions up to 80% of maximum isometric force were recorded. Contractions were divided into three sets of five repetitions. (A, B) Repetitions 1–5. (C, D) Repetitions 6–10. (E, F) Repetitions 11–15.

4.3.1 Achilles tendon force

Achilles tendon force was calculated by multiplying measured pedal force with the foot lever arm to heel lever arm length ratio. Foot lever arm length was

measured as the distance between the first metatarsal and the medial malleolus, and heel lever arm length as the distance between the medial malleolus and posterior bony surface of the heel. Lever arm lengths were taken as a projection along the sole of the foot when it was perpendicular to the shank. Therefore lever arms were considered to be perpendicular to the line of force. Installation of the foot to the pedal usually creates initial tension in the tendon and this tension was subsequently subtracted from AT force during the analysis phase. Thus AT force was underestimated, on average by 0.3 kN, in all studies, but a constant value subtraction does not affect tendon stiffness. AT stress was calculated by dividing force by tendon cross-sectional area.

4.3.2 Achilles tendon length

Achilles tendon length was measured using motion capture assisted US. With this method, US image coordinates can be transformed to the laboratory coordinate system (CS) for accurate calculation of AT length. To identify the myotendinous junction (MTJ) of the medial gastrocnemius (MG) muscle, a linear array US probe (Aloka 5712, Osaka, Japan) was placed in the sagittal plane over the right leg 2 cm medial to the junction separating the medial and lateral portions of the gastrocnemius muscle. An outline of the probe was drawn on the skin for later identification of the same muscle area in studies where it was necessary. An impedance-matched acoustic pad was placed under the probe to ease propagation of ultrasound waves, and the probe was secured to the leg with elastic bandages (Figure 10B). A digital camera (InLine 250, Fastec Imaging, San Diego, USA) was placed on the ankle dynamometer's left side to image movement of the probe during measurements. The camera's optical axis was perpendicular to the sagittal plane and the camera was focused on the US probe's reflective markers that were placed on the probe handle to enable movement tracking. The camera and the US unit operated at frame rates between 60 and 125 frames per second depending on study requirements. US images were stored in the imaging unit (Aloka Alpha 10, Osaka, Japan) and the digital camera's video was recorded to computer for later export. The system was synchronised via an analog-to-digital-converter, which was programmed to output a square-wave pulse. The square-wave pulse was fed into the US unit's pulse input and displayed on the monitor. At the same time, ECG detection was enabled, which created a moving vertical cursor that overlaps both the US image and the square-wave pulse. The US unit was synchronised by aligning the vertical cursor with the rising edge of the square-wave pulse. The camera was synchronised by a flashing light signal triggered by the same square-wave pulse. Maximum desynchronisation between camera and US images was inverse of the frame rate; $1/(125\ s^{-1})$ = 8 ms. AT cross-sectional area (CSA) was analyzed by taking transverse US scans 3 cm above the heel, except in study 1, where magnetic resonance imaging (MRI) was used.

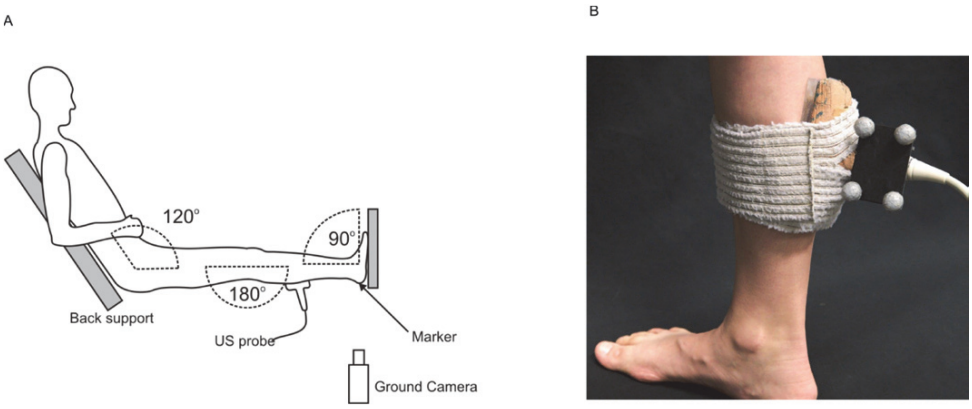

FIGURE 10 Ankle dynamometer setup. (A) Subject placement and joint angles. Note that the heel marker and ground camera that were used to record heel movement in study 1 were replaced by a potentiometer in later studies. (B) Ultrasound probe with reflective markers attached over the gastrocnemius medialis muscle of the right leg.

4.3.3 Treadmill running (paper II)

While running on a motorised treadmill (OJK-1, Telineyhtymä, Kotka, Finland), subjects wore their race shoes and the running speed was chosen according to each subject's target time. Two dimensional ankle kinematics were recorded with a high-speed digital camera (InLine 250, Fastec Imaging, San Diego, CA, USA) operating at 125 frames per second. The camera was located on the subject's right side perpendicular to the sagittal plane. Three reflective markers were placed on the subject's right leg: 1) center of the knee; 2) lateral malleolus; 3) distal head of fifth metatarsal. Markers 1-2 were attached either to the skin or running tights to minimise their movement (markers would move substantially if attached to running shorts). Marker 3 was placed on the running shoe.

To quantify running economy, subjects wore a breathing mask connected to an oxygen gas analyser (VMax series 229, Sensormedics, Yorba Linda, CA, USA). Steady state oxygen consumption was achieved by running at a constant speed for four minutes. Oxygen consumption was taken as the average during the 5th minute. Running kinematics were recorded during the last minute with a 5 second video clip containing 4–6 strides.

4.3.4 Magnetic resonance imaging (paper II)

Magnetic resonance images (MRI) of the lower right leg were taken one week before the exercise. Lever arm length and tendon CSA were analyzed from MR images, which were acquired in axial (repetition time = 4500.00, echo time = 10.90, flip angle = 90.00, matrix = 256x256 and slice thickness = 7.00 mm) and sagittal orientations (repetition time = 100.00, echo time = 3.70, flip angle = 60.00,

matrix = 256x256 and slice thickness = 5.00 mm). CSA was determined by manually outlining the AT in axial images along the tendon from the calcaneus to the MTJ of MG. The smallest CSA (located 1.7-5.1 cm from the calcaneus) was used for later calculations (Kongsgaard et al. 2005). The smallest CSA has the greatest stress concentration, and failures usually begin there during cyclic fatigue tests (Wren et al. 2003). From sagittal images, AT lever arm length was determined as the perpendicular distance from the midpoint of the superior surface of the trochlea of the talus to the line of action of AT (Maganaris, Baltzopoulos & Sargeant 2000, Finni et al. 2006). Ankle angle was 90 degrees (sole of the foot perpendicular to shank).

4.3.5 Infrared thermography (paper IV)

An infrared camera (FLIR A300, FLIR Systems AB, Danderyd, Sweden) with a focal plane array detector (size: 320 x 240 pixels; spectral range: 7.5–13 µm) and thermal sensitivity of < 0.05°C was used to assess AT skin temperature. The camera's lens was placed at a fixed distance of 0.5 m from the subject and was focused on the posterior surface of the free AT (Figure 11). The camera's recording rate was set to 0.1 Hz.

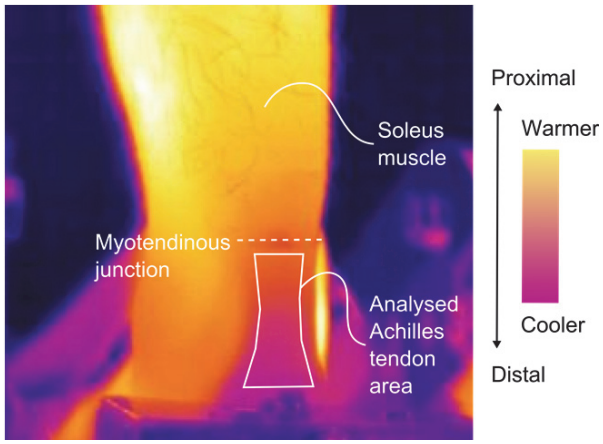

FIGURE 11 Example of thermal image analysis.

4.4 Calculations and statistics

4.4.1 Force-length curves

AT length was calculated as the distance between the MTJ of the MG muscle and proximal insertion of the AT to calcaneus. Initial position of the heel was

taken when the foot was properly installed to the pedal. Possible heel displacement in the superior-inferior direction during muscle contraction was measured with a position sensor located under the heel (or camera, as in study 1). MTJ displacement was analysed from the US images with software that exploits pyramidal implementation of the Lukas-Kanade feature tracking (Bouguet 2001). The software requires the user to place nine tracking points over the area of interest as shown in Figure 12B. The tracking points were placed just superior to the MTJ along the aponeurosis separating MG and soleus, but still on the side of MG. We have found this placement to yield the most repeatable results. The tracking algorithm has been previously shown to have a repeatability of 98% (Magnusson et al. 2003). Each trial was tracked twice and the resulting coordinates were averaged. The average was then taken as the MTJ coordinates in the US probe CS. Coordinate transformation between two CS's is always possible if both CS's are defined at all instants in time, i.e. their origins and axes are known. A rigid video calibration object was used to determine the laboratory CS, which did not change over time, and the CS of the US probe was given by the four reflective markers placed on the probe handle. The origin of the US image relative to the probe markers was determined by placing an echogenic marker on the image origin and measuring its distance from the markers. Because all movement could be restricted to the sagittal plane, analysis was performed in two dimensions and it was estimated that this introduced a ~ 0.2 mm systematic error to tendon elongation and can thus be neglected. A similar procedure has also been used in 3D space (Gerus, Rao & Berton 2011, Lichtwark & Wilson 2005). Tendon elongation was calculated by subtracting initial tendon length at the onset of force production and strain was calculated by dividing tendon elongation by initial length.

To determine AT stiffness, force-elongation curves were normalized in time and then averaged resulting in one force-elongation curve per subject. AT stiffness was calculated as the slope of the least-squares line of the ascending limb of the force-elongation curve between 10% and 80% of MVC force. An exception was study 1, where stiffness was based on five ΔF and ΔL pairs at 20, 40, 60, 80 and 100% contraction strengths. Young's modulus was determined in the same way, but from the stress-strain curve.

Stored and returned elastic energies were calculated as the area under the ascending and descending limbs of the force-length curve, respectively. Dissipated energy was calculated as the difference between stored and returned energy. Hysteresis was calculated by dividing dissipated energy by stored energy.

4.4.2 Running economy (paper II)

Cost of transport (COT) was expressed in millilitres of oxygen per kilogram per kilometre (ml O2 kg^{-1} km^{-1}), which is known to be relatively independent of running speed (Cavagna & Kaneko 1977).

Ankle kinematics were recorded because they were thought to be related to possible AT stiffness changes. Digitised joint coordinates were filtered with a Butterworth low-pass filter (8 Hz) and ankle angle was calculated between the

shank segment (from the centre of the knee to the lateral malleolus) and the foot segment (from the lateral malleolus to the distal head of the 5th metatarsal), respectively. Landing and take-off were determined from the video. All 4–6 contacts were then time normalised and averaged to yield a single ankle angle trace per subject.

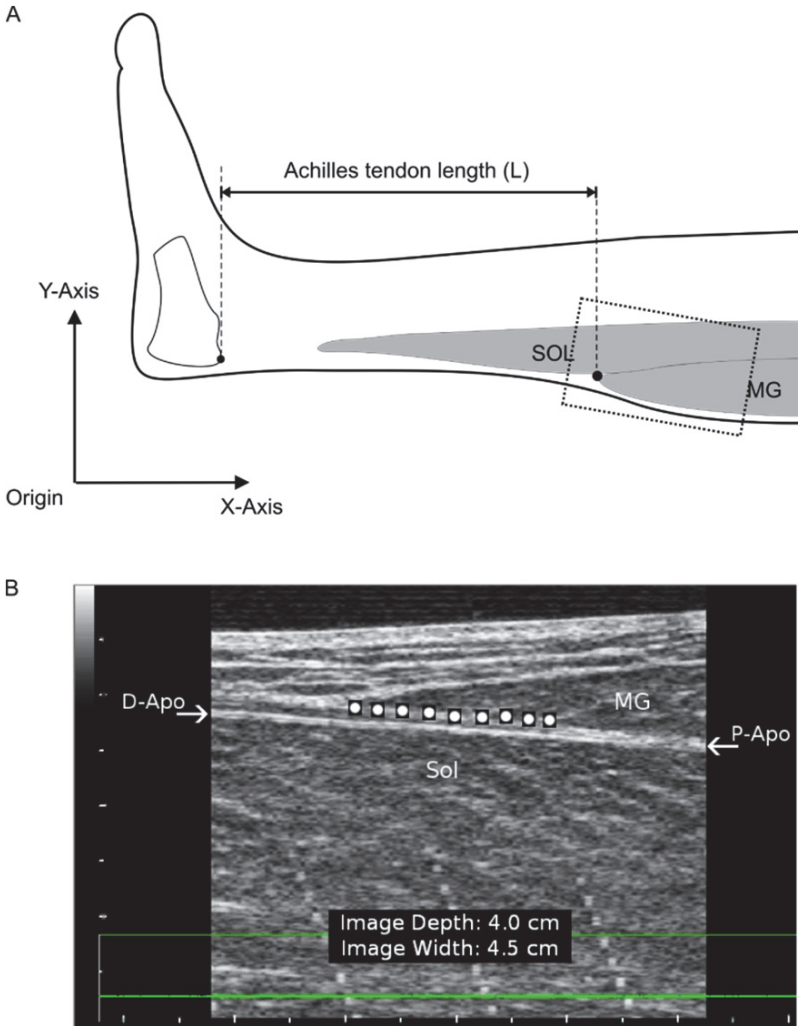

FIGURE 12 Determination of Achilles tendon (AT) length. (A) Tendon length (L) was calculated as the distance between the calcaneus and myotendinous junction of the medial gastrocnemius (MG) muscle in the laboratory coordinate system. (B) Illustration of placement of nine tracking markers over MG aponeurosis in an ultrasound image. Image B approximately corresponds to the dotted box in image A, except that the vertical axis is inverted. D-Apo = distal aponeurosis head; P-Apo = proximal aponeurosis head. Sol = soleus muscle.

To define landing technique, the angle at ground contact was offset corrected and deviations in ankle angle were determined relative to this zero value: a positive angle corresponded to dorsiflexion and a negative angle to plantarflexion. Subjects were categorised to three groups: forefoot contact, midfoot contact and heel contact. The categorisation was based on the ankle angle deflection (relative to ground contact) at the moment corresponding to 0.225 times the interval from the ground contact to the maximum dorsiflexion deflection (illustrated by circles in Figure 15). If the deflection was more than +1 degrees (dorsiflexion), it was a forefoot contact; if the deflection was less than -1 degrees (plantarflexion), it was a heel contact; if the deflection was between -1 and +1 degrees, it was a midfoot contact. Categorization was done before and after the marathon.

4.4.3 Thermal analysis and absorbed heat (paper IV)

AT temperature analysis was performed on a series of thermography images acquired during the testing (Figure 11). Average temperature over the area covering 60–80% of the free tendon length was calculated throughout the recording period in 10 second intervals. The period during which the contractions were performed was excluded from the analysis because the AT did not remain stationary. The increase of AT skin temperature was determined individually as the difference between the 1st image frame (prior to contractions) and the maximum temperature, which was usually attained 3–7 minutes after the 1st contraction. The increase of AT skin temperature was divided by the number of contraction cycles to yield average increase of temperature per cycle.

AT temperature gradients were analyzed from thermal images by dividing AT into three regions of equal size. This was done to determine whether AT temperature values were influenced by heat dissipated from proximal muscles. Average temperature of each region is denoted by T_1, T_2 and T_3, and average location by x_1, x_2 and x_3, where numbers 1, 2 and 3 indicate proximal, middle and distal regions, respectively. Temperature gradients were calculated as

$$g_i = \frac{T_{i+1} - T_i}{T_{i+1} - T_i}$$

where *i* has values 1 and 2.

Absorbed heat was calculated using the relation between absorbed heat and increase of temperature [Equation (9)]. AT mass was calculated assuming that the geometrical shape of the AT is a thin cylindrical rod with a cross-sectional area *A* and length *l*. In which case Equation (9) transforms to

$$\Delta Q = \rho A l c \Delta T \quad (10)$$

where ρ is density of the tendon. Cross-sectional area and length were taken individually, and tendon density and specific heat capacity were taken from the literature (IT'IS Foundation 2013). Below is an example of the calculation of AT absorbed heat [Equation (10)]

$$\Delta Q = 1525\ \mathrm{kg\ m^{-3}} \times 62.0\ \mathrm{mm^2} \times 175\ \mathrm{mm} \times 2372\ \mathrm{J\ kg^{-1}\ {}^{\circ}C^{-1}} \times 0.045{}^{\circ}\mathrm{C} = 0.9\ \mathrm{J}$$

4.4.4 Statistics

Normal distribution of data was tested with Kolmogorov-Smirnov test and non-parametric Wilcoxon's signed-rank test was used to test differences between sessions and groups accordingly. Spearman's linear correlation coefficient was also calculated between selected parameters. In study II, all marathon runners were treated as one group because full and half marathons were considered to be equally stressful for each individual relative to his/her training background. Data are presented as mean ± SD unless otherwise mentioned.

5 RESULTS

An example of a linear Achilles tendon force-elongation relation is shown in Figure 13. Data are from a single subject in study IV, and are based on the data shown earlier in Figure 9. The figure consists of three curves and each curve is an average of five consecutive contractions as shown in Figure 9.

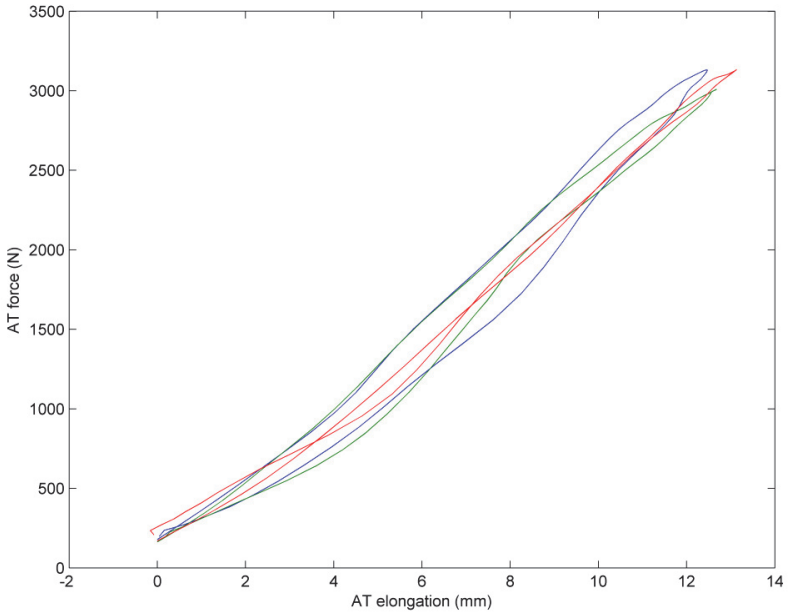

FIGURE 13 Example of Achilles tendon (AT) force-elongation curves from the same subject in study IV. Each curve is an average of five contractions.

5.1 Exercise-induced tendon fatigue (papers I and II)

The effect of exercise on tendon stiffness was investigated in papers I and II. The results are summarized below.

5.1.1 Exercise duration and intensity

During hopping until exhaustion (paper I) subjects performed on average of 1885 hops (min = 1150, max = 2 600) at a hopping frequency of 2.0–2.5 Hz. During a marathon (paper II) it was estimated, based on the speed and stride rate during treadmill running, that runners took on average 10 200 strides during a half marathon (min = 8 700, max = 12 100) and 19 200 strides during a full marathon (min = 17 200, max = 21 200). There was no significant difference in stride rate when determined on a running treadmill before and after the race (pre-race vs. post-race: 1.4 ± 0.1 Hz vs. 1.5 ± 0.1 Hz; $P = 0.204$). Racing stride rate was assumed to be the same as on the treadmill, because there was no significant difference between the average race speed of 11.2 km h^{-1} and the treadmill speed of 10.9 km h^{-1} ($P = 0.115$).

TABLE 3 Calculated maximum tensile Achilles tendon force before and after exhaustive hopping exercise (paper I) and marathon running (paper II). Note that subjects are not the same between papers I and II.

	Hopping bout (paper I) Tendon force (kN)		Marathon (paper II) Tendon force (kN)		
Subject	Pre	Post	Pre 2d	Pre 2h	Post 1h
1	3.8	3.1	2.2	3.4	3.3
2	3.2	2.8	2.1	2.6	2.5
3	3.3	2.9	3.3	3.4	3.2
4	2.7	1.2	3.4	3.7	3.6
5	4.4	3.4	3.9	5.4	5
6	3.4	2.5	2.2	2.3	2.3
7	4	3.7	2.2	–	1.3
8	4.1	3.4	3.7	4.2	3.9
9	4	3.1	2.9	3.1	1.2
10	2.4	2.2	3.5	4.4	3.5
			2.7	2.8	2.5
			2.3	2.4	2.3
mean	3.5	2.8	2.9	3.4†	2.9
s.d.	0.6	0.7	0.6	0.9	1.0
c.v.	0.17	0.24	0.22	0.26	0.36

s.d. = standard deviation; c.v. = coefficient of variation; † = Mean is not comparable to pre 2d or post 1h because number of subjects is different in pre 2h test.

5.1.2 Maximum force production

There was a significant reduction in maximum force production capacity after both exercises (
). In paper I, maximum tensile force in the AT during MVC contraction decreased by 20 ± 13% (N = 10, P < 0.01). A similar reduction was observed in paper II, where AT force decreased by 12 ± 17% between pre 2h and post 1h (N = 11, P < 0.01). Force was also 17 ± 16% lower at pre 2d than pre 2h, probably indicating a learning effect (N = 11, P < 0.001).

5.1.3 Tendon stiffness

AT stiffness did not change significantly after a hopping exercise bout or after the marathon (**Error! Reference source not found.**). Stiffness before a hopping exercise bout was 430 ± 200 N mm^{-1} and was reduced to 390 ± 190 N mm^{-1} afterwards, but the difference was not significant (N = 10, P = 0.264). AT stiffness before and after the marathon was 197 ± 62 N mm^{-1} and 206 ± 59 N mm^{-1}, respectively (N = 12, P = 0.312).

TABLE 4 Achilles tendon stiffness before and after an exhaustive hopping exercise bout (paper I) and marathon running (paper II). Note that subjects are not the same between papers I and II.

	Hopping bout (paper I) Stiffness (N/mm)		Marathon (paper II) Stiffness (N/mm)		
Subject	Pre	Post	Pre 2d	Pre 2h	Post 1h
1	571	678	227	255	291
2	118	160	188	209	194
3	690	650	267	260	283
4	231	183	310	237	252
5	610	569	168	219	246
6	650	334	179	173	165
7	337	339	132		151
8	429	355	241	261	254
9	453	433	124	159	138
10	208	188	253	297	201
11			154	153	191
12			119	116	109
mean	430	389	197	213†	206
s.d.	191	181	59	53	56
c.v.	0.44	0.47	0.30	0.25	0.27

s.d. = standard deviation; c.v. = coefficient of variation; † = Mean is not comparable to pre 2d or post 1h because number of subjects is different in pre 2h test.

5.1.4 Validity and repeatability of tendon stiffness

Figure 14 demonstrates a single ramp-derived stiffness as well as stiffness that was deduced from several ΔF vs. ΔL pairs over the linear strain region in paper 1. Stiffness was independent of the method, being 117 N mm^{-1} (R = 0.98, P < 0.01) when derived from a single ramp contraction and 118 N mm^{-1} (R = 0.97, P < 0.01) when derived from ΔF vs. ΔL pairs. This observation was repeated in all cases when ramp stiffness was calculated.

Tendon stiffness was considered repeatable, as a comparison between the pre 2d and pre 2h tests (paper II) revealed that AT stiffness was highly correlated (N = 11, R = 0.844, P<0.01), and that there were no statistical differences between the results: 203 ± 61 N mm^{-1} at pre 2d vs. 213 ± 56 N mm^{-1} at pre 2h (N = 11, P = 0.320). Note that subject 7 was excluded from the repeatability analysis, because their data were not available at pre 2h.

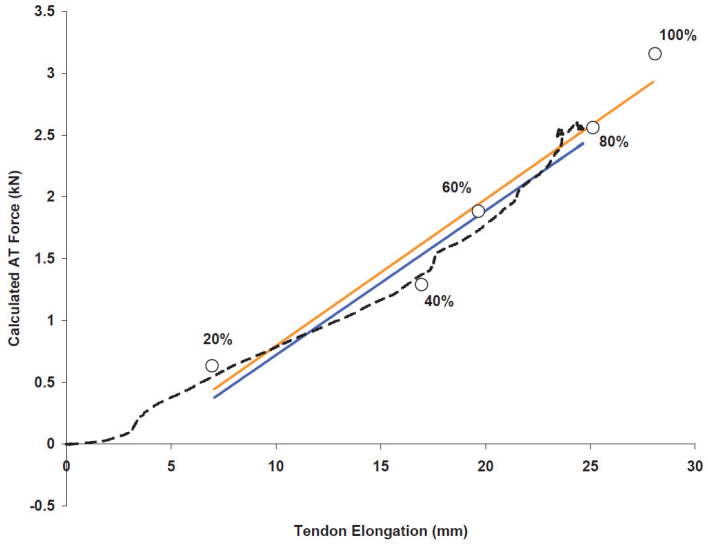

FIGURE 14 Comparison of determination of tendon stiffness with two methods (paper I): First, five ΔF vs. ΔL pairs (open circles) yield a stiffness of 118 N/mm indicated by the orange line (R = 0.97; P < 0.01). Second, a single ramp contraction (broken line) yielded a stiffness of 117 N/mm indicated by the blue line (R = 0.98; P < 0.01).

5.1.5 Running economy and landing technique

Running economy and landing technique were analyzed before and after a marathon race by running on a motorized treadmill. Results showed that average COT was 226 ± 11 ml O_2 kg^{-1} km^{-1} in the pre-race test, and significantly

higher in the post-race test, 241 ± 22 ml kg^{-1} km^{-1} (N = 12, P < 0.05). Change in stiffness did not correlate with change in COT (R = -0.262, P = 0.410).

Six out of 12 subjects changed their landing technique from forefoot contact to either midfoot contact or heel contact following the race (Figure 15). From the remaining six, three did not change their landing technique (they maintained midfoot or heel contact) and three switched from heel to midfoot contact. There were no statistical differences in any of the measured parameters between the group who used initially forefoot contact and the other subjects.

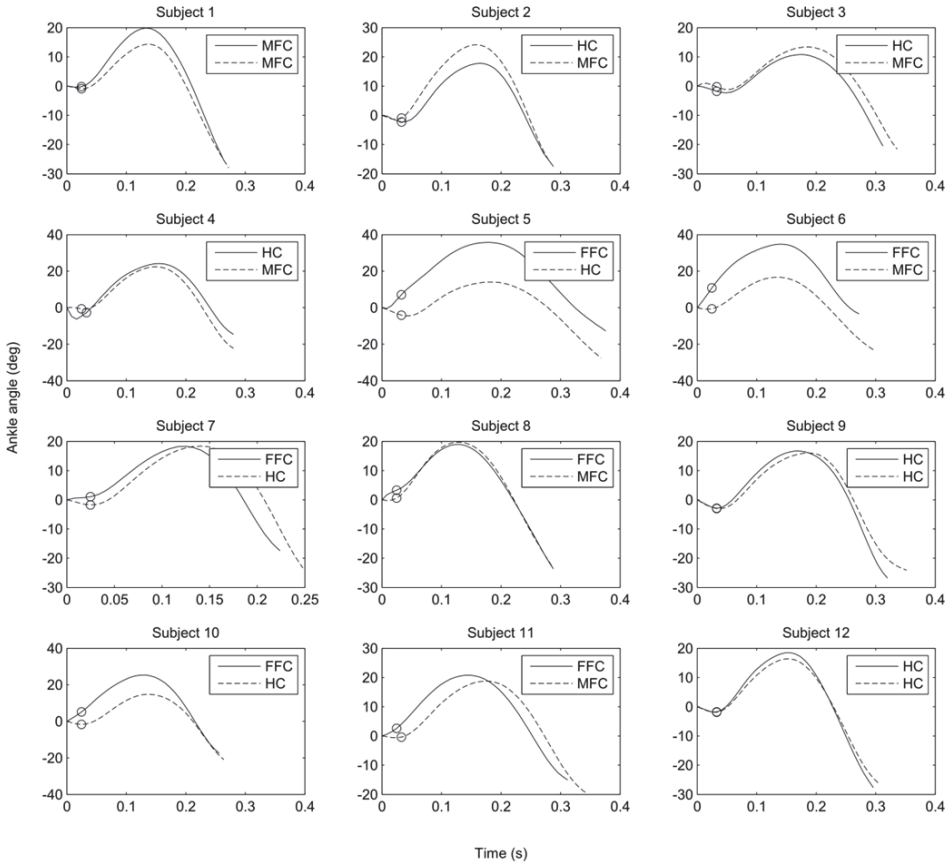

FIGURE 15 Mean ankle angle traces of 4-6 running steps before (solid line) and after (broken line) the marathon. Positive angle corresponds to dorsiflexion and negative to plantarflexion (relative to ground contact). Circles indicate the moment when ankle angle was used for categorization: FFC = fore foot contact; MFC = mid foot contact; HC = heel contact.

5.2 Stiffness vs. force

By pooling data from papers II, III and IV (and adding 2 new subjects), it was possible to analyze mechanical properties of 35 Achilles tendons. Data from paper I were excluded from this analysis, because it used slightly different methodology, and thus the results may not have been comparable to other studies. It was found that AT stiffness correlated with ankle plantar flexion force (R = 0.516, P < 0.01) and with the calculated AT force (R = 0.555, P < 0.001) during isometric MVC contraction.

Young's modulus of the AT was on average 2.0 ± 1.0 GPa in paper 1 and 1.0 ± 0.4 GPa in papers 2–4.

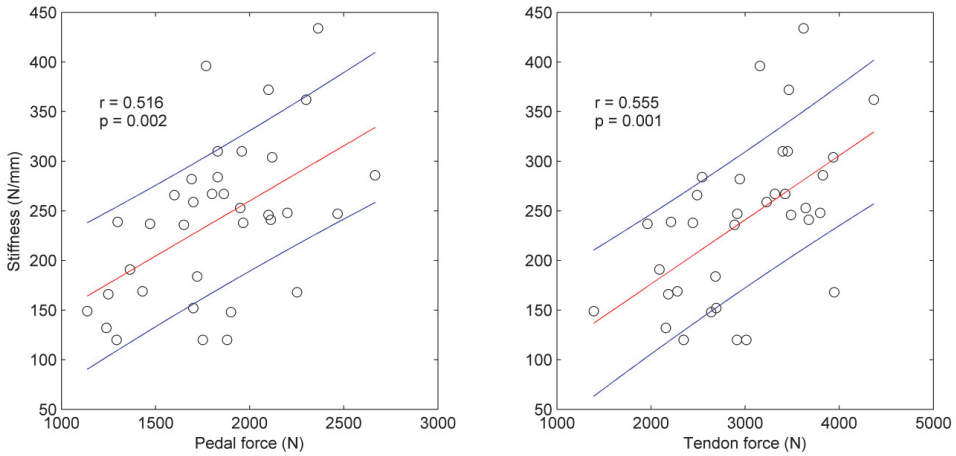

FIGURE 16 Correlation between Achilles tendon stiffness and ankle plantar flexion force (Pedal force) and calculated AT tensile force (Tendon force) during isometric maximum voluntary contraction. The red line indicates the line of best fit and blue lines indicate 50% confidence intervals.

5.3 Tendon viscoelastic properties (papers III and IV)

Tendon viscoelastic properties such as hysteresis, strain rate dependency and absorbed heat were investigated in papers III and IV.

5.3.1 Strain rate sensitivity

To yield AT strain rate sensitivity, tendon stiffness and elongation were compared between fast (duration: 1 sec) and slow (duration: 7 sec) contractions (pa-

per III). Tendon strain rate during the fast contraction was significantly higher than during the slow contraction (fast vs. slow: 10.0 ± 2.6% s^{-1} vs. 1.7 ± 0.4% s^{-1}, N = 10, P < 0.05). Corresponding normalized AT loading rates (rate of change of force divided by MVC force) were 120 ± 6% s^{-1} and 21 ± 1% s^{-1} during the fast and slow contractions, respectively (N = 10, P < 0.05). However, there were no significant differences in tendon stiffness between the fast (193 ± 22 N mm^{-1}) and slow (207 ± 18 N mm^{-1}) strain rates (N = 10, P = 0.105), nor were there significant differences in tendon elongation between the fast and slow rates at any of the force levels between 10–80% of MVC (Figure 17).

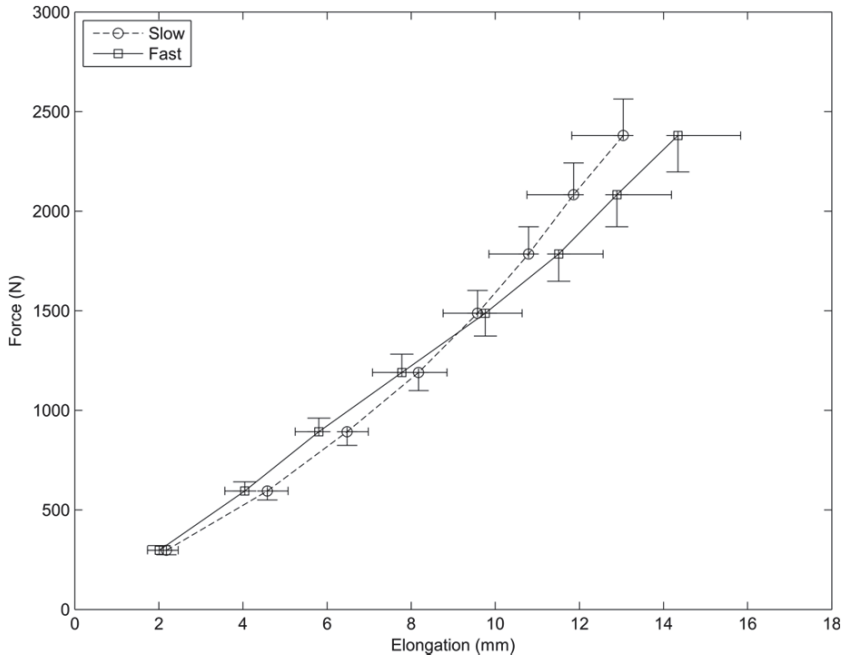

FIGURE 17 Average Achilles tendon force-elongation curves at the fast (continuous line) and slow (broken line) loading rates (N = 10). There were no statistical differences in tendon elongation between the fast and slow rates at any of the force levels from 10 % to 80 % of MVC. Lengths of the error bars indicate standard errors.

5.3.2 Hysteresis

Analyzing a total of 35 AT load-deformation curves from studies III and IV (plus additional 2 new subjects) yielded an average hysteresis of 9 ± 10% (interquartile range = 9%). A histogram of AT hysteresis is shown in Figure 19.

TABLE 5 Achilles tendon hysteresis, dissipated energy, increase of temperature and absorbed heat.

Subject	*Stored energy (J)*	*Dissipated energy (J)*	*Hysteresis (%)*	*AT mass (g)*	*Increase of temperature (°C)*	*Absorbed heat (J)*
1	15	1.7	13	17	0.022	0.9
2	11	0.4	4	21	0.031	1.5
3	13	1.0	8	22	0.008	0.4
4	5	1.5	29	17	0.058	2.3
5	8	-0.2	-3	22	0.019	1.0
6	11	0.9	7	17	0.019	0.8
7	12	1.8	16	18	0.025	1.1
8	8	0.6	3	16	0.006	0.2
9	19	1.5	8	25	0.044	2.6
10	12	4.2	35	21	0.027	1.3
11	12	0.6	4	19	0.020	0.9
12	17	1.7	10	13	0.047	1.5
13	20	1.7	9	19	0.013	0.6
14	7	0.2	4	20	0.020	0.9
15	11	1.2	11	27	0.040	2.6
16	13	1.9	14	25	0.027	1.6
17	17	3.8	22	21	0.013	0.7
18	14	2.8	20	17	0.027	1.1
19	13	0.0	0	23	0.007	0.4
mean	13	1.5	11	20	0.025	1.2
s.d.	4	1.2	10	4	0.014	0.7
c.v.	0.32	0.81	0.86	0.18	0.57	0.60

5.3.3 Hysteresis vs. increase of temperature

The main results of paper 4 are shown in TABLE 5 and Figure 18. Mean AT hysteresis was 11 ± 10% and increase of AT skin temperature, assessed with IRT imaging, was on average 0.025 ± 0.014°C per cycle (N = 19, P < 0.001; significantly different from zero). Increase of AT skin temperature during 15 stretch-shorten cycles correlated with hysteresis, which was measured simultaneously (N = 19, R = 0.471, P = 0.042).

Achilles tendon stored on average 13 ± 4 J of energy and absorbed 1.5 ± 1.2 J of heat. Dissipated heat and absorbed heat, 1.2 ± 0.7 J, were neither statistically different (N = 19, P = 0.629) nor correlated (N = 19, R = 0.239, P = 0.324). Increase of AT skin temperature did not correlate with stored strain energy (N = 19, R = -0.005, P = 0.983).

There was a significant negative temperature gradient in both proximal (g_1 = -0.056 ± 0.007°C m^{-1}) and distal (g_2 = -0.029 ± 0.010°C m^{-1}) regions of the AT indicating a decrease in temperature towards the distal end of the tendon (N =

19, P = 0.001, different from zero). However, temperature gradients did not change significantly throughout the monitoring period as compared to the 1st thermography image.

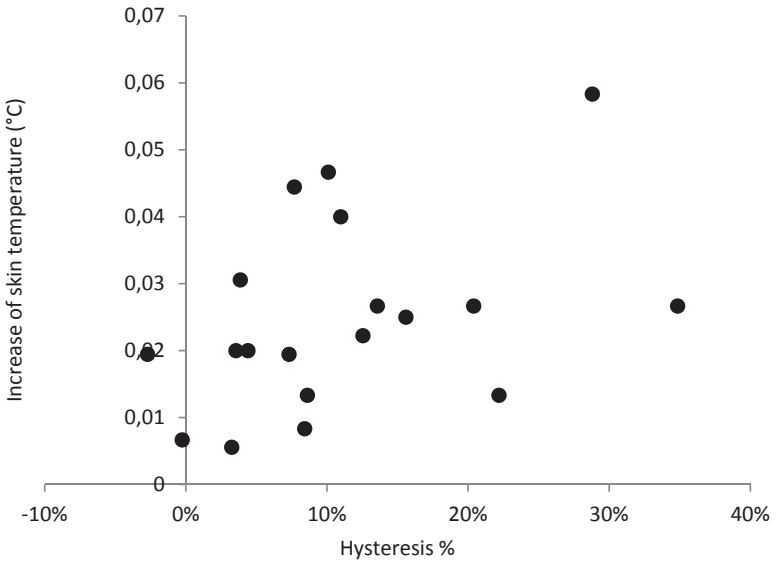

FIGURE 18 Relation between Achilles tendon hysteresis and increase of skin temperature.

6 DISCUSSION

The main findings of the current thesis were: 1) AT stiffness was unchanged after a high-intensity two-legged hopping exercise bout and after a long-duration marathon run. Although no changes were observed in AT stiffness, maximum ankle plantar flexion force was decreased after both exercises. In addition, marathon running induced a reduction in running economy and changes in running technique, whereby the most prevalent change was a switch from forefoot contact to midfoot or heel contact; 2) AT did not demonstrate strain rate sensitivity, but did demonstrate hysteresis, which was accompanied by an increase in AT skin temperature during 15 stretch-shorten cycles. Furthermore, individual variation in AT hysteresis was correlated with increase of AT skin temperature: higher hysteresis was related to a higher increase of temperature.

6.1 Exercise-induced tendon fatigue

Tendon fatigue was quantified as a reduction in AT stiffness after two-legged hopping exercise until exhaustion and after a marathon run. Two-legged hopping was considered more intense because of the higher frequency, 2.0–2.5 Hz, compared to 1.3–1.6 Hz during a marathon, whereas the marathon was longer in duration. Two-legged hopping exercise included an average of 1885 hops, and the marathon included an estimated 10 200 or 19 200 steps during the half or full race, respectively.

Both exercises induced a reduction in maximum ankle plantar flexion force. Maximum force decreased by 20% after two-legged hopping and 12% after the marathon. A greater reduction in maximum force after two-legged hopping exercise may be explained by shorter recovery time after hopping. Maximum force was measured 5–10 minutes after the end of the hopping bout, but 1–2 hours after the marathon. It is also notable that there were large inter-individual deviations. Minimum and maximum decreases in force after hopping were 8% and 56%, respectively. After a marathon, the corresponding numbers were 0% and 61%.

Despite a reduction in maximum force generating capacity of the muscles, there was no reduction in AT stiffness after either exercise. The findings are in agreement with previous in vivo studies of human tendons that have not found a decrease in tendon stiffness after concentric contractions (Ullrich, Mademli & Arampatzis 2009, Mademli, Arampatzis & Walsh 2006) or after a 30-min or 5k run (Farris, Trewartha & McGuigan 2011, Lichtwark, Cresswell & Newsham-West 2013). By contrast, some reports demonstrate that isometric contractions were able to increase VL tendon compliance (Kubo et al. 2001a, Kubo et al. 2001b, Kubo, Kanehisa & Fukunaga 2005), thus suggesting that lower frequency loading may be more damaging to tendon. Interestingly, Wang et al. (Wang, Ker & Alexander 1995) showed the opposite; fatigue ruptures occur over a shorter duration than would be predicted from creep ruptures alone. As creep and fatigue failures are two distinct phenomena (Wang, Ker & Alexander 1995), and a repeated isometric fatigue test may actually be a fatigue test superimposed on a creep test, this may explain why VL tendon stiffness decreased after repeated isometric contractions but AT did not during the current studies. Another possibility is that there is a difference in AT and VL tendon resistance to fatigue.

Although some mechanical properties of human tendons are similar (Wren et al. 2001b), fatigue resistance may vary between different tendons. For example, high-stressed animal tendons showed higher fatigue resistance than low-stressed tendons despite an equivalent Young's modulus (Pike, Ker & Alexander 2000, Ker, Wang & Pike 2000). It has been hypothesized that tendons with greater stress-in-life consist of higher quality material and are therefore more fatigue resistant than tendons with lower stress-in-life (Ker, Wang & Pike 2000, Pike, Ker & Alexander 2000). Estimated maximum AT stress is 70–80 MPa (Lichtwark & Wilson 2005), whereas in patella tendon it is 30–40 MPa (Hansen et al. 2006, Westh et al. 2008). This supports the idea that AT is a higher stressed tendon than the VL tendon and may explain the difference between their fatigue resistances.

Looking closely at the only specimen data available for human AT, it shows that AT can be ruptured at physiological stress levels (40–60 MPa) after 1000 cycles or less if 8% initial strain level is exceeded (Wren et al. 2003). The current study did not measure AT strain during hopping or running, but it has been estimated elsewhere that AT strain is 8% during one-legged hopping and 3.5% during moderate pace endurance running (Farris, Trewartha & McGuigan 2011). As strain in the free tendon is higher (even double) the strain in tendon and aponeurosis combined (which is typically measured in vivo; Magnusson et al. 2003, Finni et al. 2003), there is a good chance that the 8% limit was exceeded in the free tendon. Thus the strain conditions set by Wren et al. (Wren et al. 2003) to rupture AT with less than 1000 cycles were probably met, at least in some individuals, during the current studies.

As AT does not seem to be nearly as prone to fatigue damage as specimen studies suggest (Wren et al. 2003), the reason for early ruptures in tendon specimens may lie in the methodology. Wren et al. (Wren et al. 2003) note that the

AT is especially hard to work with and non-uniform strains often occur. Furthermore, comparisons are difficult because there is not always a clear indication of tendon strain during fatigue tests (Pike, Ker & Alexander 2000, Wang, Ker & Alexander 1995). Another disadvantage of cadaver tendons is that they are usually extracted from older donors and therefore suffer from possible tissue degradation and decreased stiffness due to ageing (for review of ageing see Reeves 2006).

The effects of fatiguing exercise on joint kinematics were measured after the marathon, but not during hopping. During two-legged hopping it was merely visually checked that subjects maintained a forefoot landing throughout the exercise (they wore no shoes, which made observations easier). During the marathon, subjects wore their running shoes and their landing technique was quantified on a running treadmill. It was found that contact time and stride rate did not change after the race, but changes in landing technique were observed. Half of the runners landed initially with the forefoot, but they all switched to midfoot or heel contact after the race. The other half either did not change landing type or changed from midfoot to heel contact. Measuring post-marathon oxygen consumption simultaneously with running technique revealed that COT (ml O^2 kg^{-1} km^{-1}) increased by 7 % when measured at race speed. This indicates a reduction in running economy, which is in line with previous observations, although the mechanism(s) of decreased running economy have not been identified (Kyröläinen et al. 2000, Hausswirth, Bigard & Guezennec 1997). It is concluded that switching from forefoot to midfoot or heel contact during the current study was probably caused by muscle fatigue, as indicated by a reduction in maximum plantar flexion force. Muscle fatigue-induced changes in running technique may have caused the decrease in running economy.

Exercise-induced overload has been suggested as a predisposing factor for AT overuse injuries (Järvinen et al. 1997). Against this background, it is interesting to note that a large body of evidence suggests that physical activity does not reduce the stiffness of the AT, but rather a lack of physical activity does. A typical training effect, regardless of whether training is plyometric or isometric resistance training, is an increase in AT stiffness (Burgess et al. 2007), although the effect may be invariant to training background as runners and non-runners were found to have similar AT stiffness (Rosager et al. 2002). There is strong evidence that a certain magnitude of strain must be exceeded to trigger tendon training adaptations (Arampatzis et al. 2010). In contrast to physical activity, it is well documented that physical inactivity, such as 90 days of bed rest (Reeves et al. 2005) or aging-related reductions in exercise frequency (Narici & Maganaris 2006), decrease AT stiffness. When the training data are supplemented with the finding that neither of the current exercises nor previous exercises (Farris, Trewartha & McGuigan 2011, Mademli, Arampatzis & Walsh 2006, Lichtwark, Cresswell & Newsham-West 2013) caused a reduction in AT stiffness, the theory of overuse-induced mechanical changes in the human AT is not strongly supported by the published literature.

6.2 Achilles tendon viscoelastic properties

The current data indicate that AT stiffness and elongation were not different between the fast and slow strain rates. This is in line with the majority of previous in vitro studies indicating time-independent mechanical behavior (Abrahams 1967, Ker 1981, Mabuchi, Hayatsu & Fujie 1991, Noyes, Delucas & Torvik 1974, Wang, Ker & Alexander 1995, Woo et al. 1990, Wren et al. 2001a, Herrick, Kingsbury & Lou 1978). To our knowledge, only two studies have investigated tendon strain rate dependency in vivo. Both studies found that AT (Gerus, Rao & Berton 2011) or patella tendon (Pearson, Burgess & Gladys N.L. Onambele 2007) strain decreased as strain rate increased.

Early failure experiments demonstrated that ligaments were more prone to ruptures at high strain rates than at low strain rates (Crowninshield & Pope 1976, Noyes, Delucas & Torvik 1974). Because later studies did not find such a relationship (Danto & Woo 1993, Woo et al. 1990, Ng et al. 2004, Wren et al. 2001a, Wren et al. 2001b), it has been postulated that tendon viscoelastic properties may vary (Wren et al. 2001a). The finding that high-stressed flexor tendons have a lower hysteresis than low-stressed extensor tendons supports this idea (Shadwick. 1990). As high-stressed tendons have higher fatigue resistance than low-stressed tendons (Pike et al. 2000), they possibly consist of different material (Ker et al. 2000). This may explain why AT in the current study did not exhibit strain rate sensitivity but the patella tendon did in a previous study (Pearson, Burgess & Onambele 2007).

The current strain rate range was 1.7–10% s^{-1}. This is comparable to previous values of 1–10% s^{-1} in AT specimens, within which AT was also found to be insensitive to strain rate (Wren et al. 2001a). During movement AT strain rates may be higher. It seems that AT strain rates in this thesis are lower than the reported 15–70% s^{-1} in running (Farris, Trewartha & McGuigan 2011, Lichtwark, Bougoulias & Wilson 2007), but comparable to those of walking (Lichtwark, Bougoulias & Wilson 2007). The quite substantial strain rate range (6-fold difference), without any noticeable changes in tendon stiffness or elongation, indicates that AT mechanical behavior may be strain rate independent throughout the physiological strain rate range. In excised tendon, even strain rates from 5% s^{-1} to 100% s^{-1} were not able to induce changes in tendon stiffness (Herrick, Kingsbury & Lou 1978).

The results showed, as hypothesized, that AT demonstrated hysteresis of 9% and hysteresis was positively correlated with increase of AT skin temperature. As larger hysteresis was related to a larger increase of temperature, this indicates that variation in force-length derived AT hysteresis may be of physiological origin. To confirm that the increase of AT temperature was not due to heat transferred from the distal muscles that had to work to stretch the AT, temperature gradients of the free AT were analyzed throughout the monitoring period. The results showed that there were no changes in temperature gradients in the proximal or distal regions of the free AT. The lack of heat transfer from proxi-

mal muscles to the distal tendon was also supported by the absence of a correlation between stored strain energy, which equates to the work done by the muscles, and increase of AT skin temperature.

In study IV, AT stored an average of 13 J of elastic strain energy per contraction. This is much lower than 51 joules estimated during one legged-hopping (Lichtwark & Wilson 2005). One-legged hopping is a particularly stressful task for the AT, as it induces twice as much strain as running (Farris, Trewartha & McGuigan 2011). Since stored strain energy is related to the second power of elongation [Equation (2)], doubling the strain quadruples the stored energy. This explains how AT could have stored four times the energy in one legged-hopping compared to the current study.

The maximum work done by a muscle in a single contraction is 120 J kg^{-1} (Alexander 2003). The triceps surae muscle group has a mass of 450 grams (Ward et al. 2009). Thus it is able to do a maximum of 54 joules of work per hop, which is comparable to the stored energy of the AT during one-legged hopping, 51 joules (Lichtwark & Wilson 2005). Thus AT energy storage during one-legged hopping is likely to require maximum muscle work, which can only be sustained for a short period of time. In comparison, AT energy values in the current study are more likely to be similar to those during sustained activities such as endurance running.

The relation between dissipated energy and absorbed heat in the AT was also investigated. It was postulated that if force-length derived hysteresis is correct, dissipated heat will be similar to absorbed heat, which was estimated independently from IRT data. The hypothesis was confirmed, as dissipated heat was 1.5 J and equal to absorbed heat, 1.2 J. However, there was no correlation between dissipated energy and absorbed heat. This indicates that the two are not related. The lack of a correlation may indicate that some of the assumptions made in the AT calculations were unrealistic. For example, AT was assumed to be a cylindrical rod with a constant cross-sectional area, and increased AT skin temperature was assumed to represent the mean increase of temperature over the whole AT volume.

We calculated the power at which AT dissipates mechanical energy as heat during running. We used values from the current work, 1.5 J as the dissipated energy and 20 g as the AT mass, and assumed a stride rate of 1.5 Hz. Thus each AT converts elastic strain energy to heat at a power of 113 W kg^{-1}. If running speed is 10 km h^{-1} and COT is 4 kJ kg^{-1} km^{-1}, chemical energy is consumed at a rate of 11 W kg^{-1}. Assuming that 20% of body mass is muscle that works at a given instant, chemical energy is converted to heat at a rate of 56 W kg^{-1} in the muscle. This is an approximation, but tells us that due to hysteresis, AT may convert mechanical energy to heat at rates that are comparable to chemical energy conversion rates in the muscle. As dissipation of energy to heat raises the temperature of a tendon in both animals (Birch, Wilson & Goodship 1997) and humans (Farris, Trewartha & McGuigan 2011), and excessive increases in temperature, known as tendon hyperthermia, may damage the tendon

(Wilson & Goodship 1994), vigorous or long-lasting exercise may predispose AT to heat-related injuries.

With the current results we were able to show an increase of AT skin temperature after 15 contractions with IRT imaging. Because the loading ceased after 15 contractions, it is not known what happens to temperature during continuous exposure to cyclic stretching and shortening. It is possible that the currently observed increase of temperature continues to reach hyperthermic values, but it is equally possible that the increase of temperature levels off due to thermoregulation, and hyperthermic temperatures are never reached. As larger hysteresis was related to a larger increase of temperature, some tendons may be more prone to hyperthermia than others. Since tendon hyperthermia was not demonstrated in the current study, this idea remains to be tested in future studies.

6.3 Methodological considerations

The current maximum plantar flexion force was 2.5 times body weight. This roughly equates to peak ground reaction force at endurance running pace. At sprint running pace, ground reaction forces are higher, around 3.0 times body weight. It is possible that the currently achieved MVC force of 2.5 times body weight was a slight underestimation. This could be due to learning and suboptimal muscle fiber length. Learning was demonstrated in paper II before the marathon. Maximum plantar flexion force was increased by 17 % between the first testing session (pre 2d) and the second session (pre 2h). The second factor that may have decreased ankle plantar flexion force is the ankle angle. We have observed that the best results in our ankle dynamometer are typically achieved in slight dorsiflexion. The effect is not huge but distinguishable. In slight dorsiflexion (around 5 degrees) MVC force increases by 10–15% compared to the 90 degree ankle angle. This is thought to be due to longer and thus more optimal sarcomere lengths (Gordon, Huxley & Julian 1966). The current study used a 90 degrees ankle angle because the effect of ankle angle on sarcomere length could be individual and the forces achieved with the current setup were enough to extend the AT to the linear region (Figure 13).

The current maximum calculated AT tensile force was 3–3.5 kN. This is in line with forces previously acquired with either the optic fiber technique or buckle transducers. AT force has been reported to be 1–3 kN in walking (Finni et al., 1998), approximately 4 kN in hopping (Fukashiro et al., 1995) and slightly lower than 9 kN in running at 16 km h^{-1} (Komi et al., 1992). However, the current maximum AT force was probably a slight underestimation. In addition to the explanation above, the current AT force was underestimated, on average by 0.3 kN, because initial tensile force was always subtracted from the active force during muscle contractions. Thus it can be estimated that in optimum conditions, the current subjects may have been able to load their AT up to 3.5–5 kN.

This is in accordance with loads achieved during one legged-hopping (Lichtwark & Wilson 2005).

The current mean AT stiffness varied from 240 to 430 N mm^{-1} and Young's modulus from 0.8 to 2.0 GPa between papers II to IV and paper I, respectively. The difference may be explained by different methodology. In paper I, MTJ junction displacement was tracked manually, as opposed to automated tracking, and CSA's were acquired using MRI, as opposed to US images in studies II to IV. These differences may have led to lower AT strains as well as CSA's, and thus to higher stiffness and Young's modulus in paper I.

The current AT stiffness and Young's modulus values are in line with previously reported values. Published in vivo AT stiffness values range from 150 to 306 N mm^{-1} (Farris, Trewartha & McGuigan 2011, Lichtwark & Wilson 2005, Maganaris & Paul 2002, Foure, Nordez & Cornu 2010, Rosager et al. 2002) and Young's modulus from 0.9 to 1.2 GPa (Maganaris & Paul 2002, Lichtwark & Wilson 2005). In comparison, Young's moduli of human specimens range from 0.7 to 1.1 GPa (Schechtman and Bader, 1994; Johnson et al., 1994; Wren et al., 2001).

AT stiffness is low at low forces, but increases towards high forces, as shown in Figure 5. Yet there is no agreement about the range within which AT stiffness should be measured. Stiffness is often taken as the slope of the line between 10–100% of maximum AT force (Maganaris & Paul 2002, Lichtwark & Wilson 2005), but also ranges such as 90–100% have been reported (Rosager et al. 2002). This discrepancy may explain some of the differences observed between studies. In the current thesis, varying stiffness was accounted for by measuring stiffness within the same range in pre- and post-exercise measurements.

The current data showed that coefficient of variation of AT stiffness was 0.33. Because a test-retest comparison (paper II) showed that the correlation coefficient was significant and mean values were not significantly different, it seems that day-to-day repeatability of AT stiffness under similar conditions is good. To investigate whether variations in stiffness are of physiological origin, correlations were performed between stiffness and several physiological parameters. Positive correlations were found between stiffness and both ankle plantar flexion force and calculated AT force during MVC (Figure 16). This has also been observed elsewhere (Stenroth et al. 2012). Thus it seems that AT strains are likely to be similar between individuals, and that those who generate higher forces ultimately have stiffer tendons.

The current mean AT hysteresis was 9%. This agrees with 5–10% hysteresis reported in tendon specimens (Ker 1981, Eliasson et al. 2007, Riemersma & Schamhardt 1985, Wang & Ker 1995, Bennett et al. 1986). The current results contrast to those previously acquired in vivo where mean AT hysteresis was more than 20% (Lichtwark & Wilson 2005, Farris, Trewartha & McGuigan 2011, Foure, Nordez & Cornu 2010, Kubo et al. 2002). The underlying reason for this difference is currently unknown. Possible physiological and methodological factors are discussed below.

Physiological adaptation of hysteresis was studied in pig flexor and extensor tendons and it was found that high-stressed flexor tendons have a lower hysteresis than low-stressed extensor tendons (Shadwick 1990). This indicates that hysteresis may depend on the loading history of the tendon. In humans, understanding of tendon adaptations has been mainly acquired through studies of tendon stiffness. These data show that stiffness increases during maturation (Waugh et al. 2011) and after training (Westh et al. 2008), and decreases during aging (Narici & Maganaris 2006) and due to inactivity (Reeves et al. 2005). However, there is usually no clear evidence of causality; for example, an age-related decrease in stiffness may be a consequence of the aging process, but it may also be caused by decreased physical activity and loss of muscle force, suggesting that tendon adapts to its stress history (Stenroth et al. 2012). Adaptation in tendon hysteresis is less studied. One study investigated the effects of plyometric training (hopping and jumping) on AT hysteresis and found that hysteresis decreased without any significant change in AT CSA (Foure, Nordez & Cornu 2010). Similar findings were reported after 3 weeks of stretching, which also resulted in a decrease in AT hysteresis (Kubo, Kanehisa & Fukunaga 2002). Thus the current subjects may have achieved or maintained an AT that has a small hysteresis by regularly engaging in physical activity.

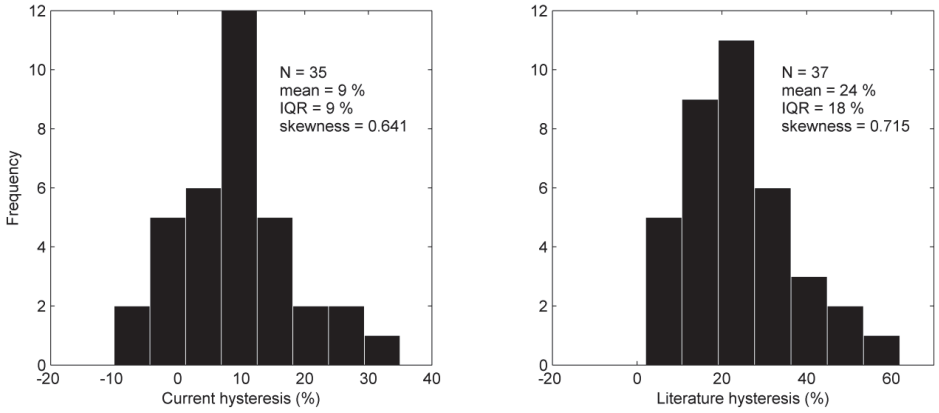

FIGURE 19 Histograms of Achilles tendon hysteresis. Data are from the current thesis (left) and from the published literature (right) (Foure, Nordez & Cornu 2010, Lichtwark & Wilson 2005, Farris, Trewartha & McGuigan 2011, Maganaris & Paul 2002). IQR = interquartile range.

Figure 19 displays two Achilles tendon hysteresis histograms, both acquired in vivo. The histogram on the left is a collection of 35 tendons from the current thesis and the histogram on the right is from 37 tendons from published literature (Lichtwark & Wilson 2005, Farris, Trewartha & McGuigan 2011, Foure, Nordez & Cornu 2010, Maganaris & Paul 2002). In both histograms, data are scattered around the mean value and the right tail is a bit longer than the left

tail (measured by skewness). The longer right tail may indicate biased results. This could be achieved if negative results are neglected. As negative hysteresis indicates that tendon does positive work during a stretch-shorten loop, negative values could be considered "wrong" (Finni et al. 2012). The underlying problem in deducing tendon hysteresis in vivo is that the standard deviation is typically around 10% (Lichtwark & Wilson 2005, Farris, Trewartha & McGuigan 2011, Foure, Nordez & Cornu 2010, Kubo et al. 2002), which is higher than the true mean, 7%, reported in tendon specimens. Thus it is important in future to improve the accuracy of the determination of human tendon hysteresis in vivo.

6.3.1 Known limitations

The current AT force was calculated based on the measured lever arm lengths. Foot lever arm length depends on the point of force application. In the current studies, the point of force application was assumed to be under the 1st metatarsal. This is true only at the time of maximum force. We have verified with pressure insoles that the force vector travels from the 5th metatarsal towards the 1st metatarsal when force increases - and vice versa as force decreases - thus increasing the foot lever arm. However, tendon lever arm length also increases during the application of force (Rugg et al. 1990, Maganaris, Baltzopoulos & Sargeant 2000) and these changes may cancel each other out in gear ratio calculations. Thus we have used constant lever arm lengths in our calculations. Even if a small error is introduced, it is likely to be constant throughout the experiments and does not influence the main results of the current thesis.

Due to its superficiality and pennated muscle fibers, AT is one of the most commonly studied tendons with US. However, determination of AT mechanical properties with US is associated with some limitations related to AT anatomy. AT has three independent heads, but its length change is typically taken from the MG head (Lichtwark & Wilson 2005, Farris, Trewartha & McGuigan 2011, Foure, Nordez & Cornu 2010, Maganaris & Paul 2002, Kubo et al. 2002). In addition, ankle plantar flexion torque is not transmitted by AT only, but has a contribution from deep plantar flexors (Finni, Komi & Lepola 2000, Finni et al. 2006). Thus the differential activation of synergist muscles may compromise the deduction of either AT hysteresis or stiffness in vivo. This possibility is acknowledged here, but no corrections due to varying synergist activation were made, because not enough information is available about how these corrections should be applied.

Increases in AT skin temperature were assessed with IRT imaging. A limitation of IRT imaging is that it is only able to record surface temperature. Heat transfer rate P in a cylindrical-shaped tendon between the core and the surface is given by the equation $P = 2 * pi * L * c * \Delta T$ (equation for thermal conduction), where L = tendon length (0.2 m), c = thermal conductivity (0.39 J m^{-1} K^{-1}; IT'IS Foundation 2013) and ΔT = 5°C = 5 K is the temperature difference between the tendon core and surface (Wilson & Goodship 1994). With substitution, this becomes P = 2 * 3.14 * 0.39 J m^{-1} K^{-1} * 0.2 m * 5 K = 2.45 W. This transfer rate is so fast that it would take only 10 seconds to transfer 22.5 J, the amount originally

absorbed by the tendon after 15 contractions, from the core to the surface. Thus it seems likely that the increase of temperature due to hysteresis is evenly distributed within the AT. However, it is not known whether the increase of AT skin surface temperature truly reflects the increase of AT temperature. Evidence that the two are the same was given by the observation that absorbed heat, calculated from the increase of AT skin temperature, was similar to dissipated heat, determined from the tendon force-length relation.

7 CONCLUSIONS

The main findings and conclusions of the present thesis are:

1. Achilles tendon was not fatigued after high-intensity hopping or a long-duration marathon run as evidenced by unchanged stiffness deduced from tendon force-length relation. The current findings are in agreement with the majority of previous in vivo investigations of tendon fatigue in AT, but disagree with some in vivo observations of patella tendon fatigue, and with in vitro AT fatigue studies. The different results between AT and patella tendons could be explained by a more intense stress history in the AT, which improves AT's resistance to fatigue, or by the loading model; two-legged hopping was chosen due to its high frequency, and marathon running due to its long duration, but they both allow AT relaxation between stretches, which may save the tendon from creep damage and thus increase time to fatigue. Unloading of the tendon may explain why tendon fatigue is difficult to demonstrate during natural movements despite their high frequency and long duration. These findings do not support the idea of overuse as a mechanism for tendon injuries.
2. Running on motorised treadmill revealed that running economy was reduced by 7% after marathon as evidenced by increased oxygen consumption at constant speed. Runners also changed their running technique. Prior to marathon majority of the runner made ground contact with fore foot, but switched to mid or rear foot contact after the race. Change of running technique may have been initiated by reduction in maximum isometric force generating capacity of the calf muscles, which was 12% lower after the marathon, or possibly by a defence mechanism. As fore foot contact increases loading of the ankle extensors compared to mid or rear foot contact, which increases loading of the knee extensors, runners may have adapted mid or rear foot contact during the race to protect their AT. Thus it can be speculated that humans may compromise locomotion economy to protect their tissues during vigorous exercise.
3. Achilles tendon did not demonstrate strain rate sensitivity but did demonstrate 9% hysteresis. These findings are in agreement with previous in vitro

experiments, where strain rate sensitivity has not been observed even within a strain rate range of 5–100% s^{-1} and hysteresis has been consistently found to be 5–10%. However, the current findings contradict with previous in vivo investigations of AT hysteresis, which have shown that AT hysteresis is on average as high as 24%. The difference may be explained by methodological as well as physiological variation.

4. Origin of the variation in AT hysteresis was tested by comparing AT hysteresis to increase of AT skin temperature during 15 stretch-shorten cycles. The results showed that higher hysteresis was related to higher increase of temperature. This indicates that variation in hysteresis could be of physiological origin rather than a measurement artefact. As high temperatures have been shown to be a potential risk factor for tendon damage, the current findings support the idea that tendons with high hysteresis are at risk of elevated temperatures and heat-induced injuries. However, as hyperthermic temperatures were not reached in the current study, this idea remains to be tested in future studies.

YHTEENVETO

Akillesjänteen viskoelastiset ominaisuudet ja mekaaniset vasteet yksittäiseen kuormitukseen

Akillesjänne (AJ) on ihmiskehon vahvin jänne. Se on vain sormen paksuinen, mutta voi kestää pienen henkilöauton painon. Venyessään jänne varastoi energiaa 750 J/kg, mikä on merkittävä määrä. Venyvä lihas taas varastoi energiaa 4 J/kg ja supistuva lihas puolestaan tekee työtä 120 J/kg. Tarvitaan siis 6 grammaa lihasta tekemään työ, jonka yksi gramma jännettä pystyy varastoimaan. Jänteiden ansiosta mm. juoksun taloudellisuus on kaksinkertainen ja hypyn teho kymmenkertainen verrattuna siihen mitä pelkällä lihaksella voitaisiin saavuttaa. Koska jänne on elastinen, se palauttaa 90–95 % varastoimastaan energiasta. Loput 5–10 % energiasta muuttuu lämmöksi ilmiössä, jota kutsutaan hystereesiksi. Hystereesi on osa laajempaa ominaisuutta, joka tunnetaan viskositeettinä. Koska jänteillä on sekä elastisia että viskoosisia ominaisuuksia, sanotaan että jänteet ovat viskoelastisia.

Vahvuudestaan huolimatta AJ on yksi loukkaantumisille alttiimmista jänteistä. AJ on edustettuna 40 %:ssa kaikista jänteen katkeamistapauksista. Silti AJ:n vammojen syntyperä tunnetaan erittäin huonosti. AJ katkeaa, kun sen murtolujuus ylitetään. Koska murtolujuuden on arvioitu olevan 2–8-kertainen suurimpaan fysiologiseen kuormaan verrattuna, AJ:n katkeamista on ollut vaikea selittää. Ehkäpä suosituin selitys on ollut ylirasitusteoria. Se sanoo, että ylirasittunut jänne vaurioituu, ja vaurioiden kasautuessa jänne katkeaa lopulta ehkä vain murto-osalla siitä kuormasta, jonka se terveenä kesti. Teoria on todistettu elottomilla jänteillä, muttei koskaan elävässä ihmisessä. Elottomien jänteiden rasituskokeissa on osoitettu, että jänne saadaan katkeamaan huomattavasti murtolujuutta pienemmällä kuormalla, jos jännettä venytetään toistuvasti. Tällöin puhutaan väsymismurtumasta. Koska väsymismurtumaa edeltää jänteen jäykkyyden aleneminen, voidaan jäykkyyttä käyttää jänteen väsymisen mittarina ilman tarvetta katkaista jännettä.

Tämän tutkimuksen tavoitteena oli saada tietoa joka auttaisi ymmärtämään liikunnan aiheuttamien jännevammojen syntymekanismeja. Väitöskirja koostui neljästä osaraportista, jotka on julkaistu tutkimusraportteina tieteellisissä vertaisarvioiduissa aikakausilehdissä. Kaksi ensimmäistä raporttia pyrkivät vastaamaan kysymykseen 1) väsyykö akillesjänne yksittäisen raskaan liikuntasuorituksen aikana? Mahdolliset merkit jänteen väsymisestä tukisivat ylirasitusteoriaa. Kahdessa viimeisessä raportissa tutkittiin 2) voidaanko viskoelastisilla ominaisuuksilla selittää jännevammojen syntyä? Aiempien tutkimusten perusteella suuri jännityksen muutosnopeus ja hystereesin aiheuttama jänteen lämpötilan nousu voivat olla potentiaalisia riskitekijöitä jännevammojen synnyssä.

Kaikki väitöskirjan mittaukset tehtiin laboratorio-olosuhteissa ja koehenkilöt osallistuivat mittauksiin vapaaehtoisina. Kaikissa väitöskirjan osaraporteissa määritettiin AJ:n voiman ja pituuden välinen yhteys. Voima määritettiin nilk-

kadynamometrissä, jossa mitattiin oikean nilkan ojennuksessa syntyä ulkoinen reaktiovoima, joka muunnettiin AJ:n voimaksi laskemalla. Samaan aikaan voiman kanssa määritettiin AJ:n pituus ultraäänikuvantamisen ja liikeanalyysin yhdistelmällä. Menetelmä on käytetyistä tarkin, sillä se ilmoittaa kahden pisteen välisen etäisyyden samassa koordinaatistossa eikä sisällä lainkaan korjaustermejä. Keskiarvostetuista voima-pituus-käyristä määritettiin jäykkyys nousevan osan kulmakertoimena sekä hystereesi nousevan ja laskevan käyrän väliin jäävän osan pinta-alana.

Akillesjänteen väsymistutkimuksissa jänteen rasitusmalleja oli kaksi: kahden jalan hyppely ja maratonjuoksu. Koska jänteen väsymistä ei ole aiemmin onnistuttu osoittamaan ihmiskokeissa, rasitusmallit oli valittu niin, että ne edustavat luonnollista liikkumista ja eroavat aiemmista ihmiskokeista olemalla joko korkeataajuisempia (hyppely) tai pitkäkestoisempia (maraton). Jänteen mekaanisten mittausten lisäksi juoksumatolla tutkittiin maratonjuoksun vaikutusta juoksun taloudellisuuteen ja tekniikkaan.

Akillesjänteen viskoelastisia ominaisuuksia selvitettäessä mitattiin jäykkyyden riippuvuus jännityksen muutosnopeudesta ja hystereesin sekä lämpötilan muutoksen välinen yhteys. AJ:n lämpötila mitattiin jännettä ympäröivän ihon pinnalta. Mittaamiseen käytettiin lämpökameraa, jonka kuva-anturi on herkkä kappaleiden infrapuna-alueen aallonpituuksilla lähettämälle sähkömagneettiselle säteilylle. AJ:n lämpötilan nostamiseksi tehtiin 15 oikean nilkan ojennusta 80 % voimatasolle. Tämä suoritus valittiin siksi, että haluttiin välttää muualla kehossa tuotettua lämpöä, jota väistämättä syntyisi luonnollisessa liikkumisessa ja joka mahdollisesti vaikuttaisi mittausten luotettavuuteen. Viisitoista ojennusta nostaa AJ:n lämpötilaa vain vähän (< 1 aste), mutta se voidaan helposti havaita lämpökameran sensorilla.

Ensimmäisessä väsymistutkimuksessa koehenkilöt suorittivat keskimäärin 1885 kahden jalan hyppyä. Välittömästi hyppelyn jälkeen mitattu nilkan maksimaalinen ojennusvoima oli 20 % heikompi kuin ennen hyppelyä. AJ:n jäykkyys ei muuttunut merkitsevästi hyppelyn aikana (430 N/mm ennen vs. 389 N/mm jälkeen). Toisessa väsymystutkimuksessa tutkittavat käyttivät maratonin juoksemiseen aikaa keskimäärin 3 h 45 min. Tänä aikana AJ venyi arviolta keskimäärin 19 200 kertaa. Maratonin vaikutuksesta nilkan maksimaalinen ojennusvoima heikkeni 12 %. Hyppelyn tavoin myöskään maratonilla ei ollut vaikutusta AJ:n jäykkyyteen (197 N/mm ennen vs. 206 N/mm jälkeen). Jäykkyyden toisistaan poikkeavat arvot tutkimusten välillä johtuvat eri määritystavasta. Juoksumatolla tehdyssä testissä havaittiin, että juoksijoiden hapenkulutus lisääntyi maratonin aikana 7 % ja maratonilla oli vaikutus juoksutekniikkaan: ennen maratonia 6/12 juoksijaa käytti juoksumatolla juostessaan jalkaterän etuosan kontaktia, mutta kaikki vaihtoivat jalkaterän keski- tai takaosan kontaktiin maratonin aikana. Maratonin jälkeen kukaan ei käyttänyt juoksumatolla jalan etuosan kontaktia.

Akillesjänteen viskoelastisten ominaisuuksien mittaukset osoittivat odotetusti, että AJ:n jäykkyys on sama riippumatta siitä onko jännityksen muutosnopeus 2 %/s vai 10 %/s ja että AJ:n hystereesi on keskimäärin 9 %. Lisäksi ha-

vaittiin, että viisitoista peräkkäistä venytystä nostivat jänteen lämpötilaa keskimäärin 0.375°C, tai 0.025°C per venymissykli. Lämpötilan muutos per venymissykli oli tilastollisesti verrannollinen hystereesin kanssa: suurempi hystereesi oli lineaarisesti yhteydessä suurempaan lämpötilan nousuun.

Tässä tutkimuksessa ei havaittu merkkejä AJ:n väsymistä, sillä jänteen jäykkyys ei pienentynyt kahden jalan hyppelyn eikä maratonin seurauksena. Jänteen väsymismurtumia on aiemmin demonstroitu elottomilla jänteillä fysiologisilla kuormituksilla, mutta toisaalta merkkejä väsymyksestä ei ole aiemmin löydetty yhdessäkään ihmiskokeessa. Syitä elollisen jänteen kykyyn vastustaa väsymistä voidaan etsiä sekä fysiologiasta että mittausmenetelmistä. Aiemmin on osoitettu, että jänne kestää rasituskokeessa sitä kauemmin, mitä enemmän sitä on päivittäin rasitettu. Koska AJ on ihmiskehon kuormitetuin jänne, se todennäköisesti vastustaa väsymystä parhaiten. Elottoman ja elollisen jänteen kuormitusmallit eroavat myös toisistaan siten, että elottomiin jänteisiin kohdistuu usein syklisen kuormituksen lisäksi vakiona pysyvä taustajännitys, mikä voi merkittävästi lyhentää rasituskokeen kestoa. Lisäksi elottomat jänteet ovat usein säilöttyjä ja katkeavat liitoksestaan vetojännitystä aiheuttavaan laitteistoon. Kaiken kaikkiaan elottomilla jänteillä tehtyjä kokeita on erittäin vaikea verrata ihmiskokeisiin, ja tämä voi selittää miksi niiden tuloksia ei onnistuta toistamaan luonnollisessa ympäristössä, kuten tämä tutkimus osoitti.

Juoksumattotestissä havaittiin, koehenkilöiden hapenkulutus oli 7 % korkeampi maratonin jälkeen, ja puolet juoksijoista vaihtoi askelkontaktinsa jalkaterän etuosalta keski- tai takaosalle. Koska jalan etuosan kontaktissa kuormitetaan enemmän nilkkaa, kun taas jalan takaosan kontaktissa kuormitetaan enemmän polvea, näyttäisi siltä, että juoksijat siirsivät kuormitusta nilkasta polvelle juoksun edetessä. Tekniikan muutos saattoi auttaa suojaamaan akillesjännettä väsymiseltä.

Tässä tutkimuksessa AJ:n hystereesi oli 9 % eikä jänteen jäykkyyden havaittu riippuvan jännityksen muutosnopeudesta. Tulokset ovat ristiriidattomia aiempien irrallisilla jänteillä tehtyjen tutkimusten kanssa ja vahvistavat ajatusta, että liikkeen nopeus ei itsessään ole riskitekijä jänteen katkeamiselle. Nykyiset tulokset hystereesistä ovat ristiriidassa aiempien ihmiskokeiden kanssa, joissa on havaittu, että AJ:n hystereesi on selvästi nykyistä määritettyä arvoa korkeampi, keskimäärin 24 %. Varmaa syytä tähän ei tiedetä, mutta haittana suuressa hystereesissä on se, että jänteen lämpötila voi nousta kuormituksessa vaarallisen suureksi. Tässä tutkimuksessa löydettiin viitteitä siitä, että korkean hystereesin ja suuren lämpötilan nousun välillä on yhteys. Koska jänteiden lämpötilat eivät kuitenkaan nousseet erityisen korkeiksi, on vielä liian aikaista arvioida säilyykö yhteys korkeammissa ja potentiaalisesti vaarallisissa lämpötiloissa.

REFERENCES

Abrahams, M. 1967. Mechanical behaviour of tendonIn vitro. Medical and Biological Engineering and Computing 5 (5), 433-443.

Alexander, R. & Vernon, A. 1975. The mechanics of hopping by kangaroos (Macropodidae). Journal of Zoology 177 (2), 265-303.

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

Alexander, R. M. 2003. Principles of Animal Locomotion. New Jersey: Princeton University Press.

Alexander, R. M. 2002. Tendon elasticity and muscle function. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 133 (4), 1001-1011.

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

Arora, N., Martins, D., Ruggerio, D., Tousimis, E., Swistel, A. J., Osborne, M. P. & Simmons, R. M. 2008. Effectiveness of a noninvasive digital infrared thermal imaging system in the detection of breast cancer. The American Journal of Surgery 196 (4), 523-526.

Askew, G. N. & Marsh, R. L. 1998. Optimal shortening velocity (V/Vmax) of skeletal muscle during cyclical contractions: length-force effects and velocity-dependent activation and deactivation. The Journal of Experimental Biology 201 (Pt 10), 1527-1540.

Belli, A., Kyröläinen, H. & Komi, P. V. 2002. Moment and Power of Lower Limb Joints in Running. Int J Sports Med 23 (02), 136,141.

Benedict, J. V., Walker, L. B. & Harris, E. H. 1968. Stress-strain characteristics and tensile strength of unembalmed human tendon. Journal of Biomechanics 1 (1), 53-63.

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

Biewener, A. A., Konieczynski, D. D. & Baudinette, R. V. 1998. In vivo muscle force-length behavior during steady-speed hopping in tammar wallabies. The Journal of Experimental Biology 201 (11), 1681-1694.

Biewener, A. A. 1998. Muscle-tendon stresses and elastic energy storage during locomotion in the horse. Comparative Biochemistry and Physiology. Part B: Biochemistry and Molecular Biology 120 (1), 73-87.

Birch, H. L., Wilson, A. M. & Goodship, A. E. 1997. The effect of exercise-induced localised hyperthermia on tendon cell survival. The Journal of Experimental Biology 200 (11), 1703-1708.

Bobbert, M. F., Huijing, P. A. & van Ingen Schenau, G. J. 1986. An estimation of power output and work done by the human triceps surae muscle-tendon complex in jumping. Journal of Biomechanics 19 (11), 899-906.

Bouguet, J.Y. 2001. Pyramidal implementation of the Lucas Kanade feature tracker. Description of the algorithm. Available in: http://robots.stanford.edu/cs223b04/algo_tracking.pdf.

Bramble, D. M. & Lieberman, D. E. 2004. Endurance running and the evolution of Homo. Nature 432 (7015), 345-352.

Burgess, K. E., Connick, M. J., Graham-Smith, P. & Pearson, S. J. 2007. Plyometric vs. isometric training influences on tendon properties and muscle output. Journal of Strength and Conditioning Research 21 (3), 986-989.

Carrier, D. R., Kapoor, A., Kimura, T., Nickels, M. K., Satwanti, Scott, E. C., So, J. K. & Trinkaus, E. 1984. The energetic paradox of human running and hominid evolution [and comments and reply]. Current Anthropology , 483-495.

Cavagna, G. A., Heglund, N. C. & Taylor, C. R. 1977. Mechanical work in terrestrial locomotion: two basic mechanisms for minimizing energy expenditure. The American Journal of Physiology 233 (5), R243-61.

Cavagna, G. A. & Kaneko, M. 1977. Mechanical work and efficiency in level walking and running. The Journal of Physiology 268 (2), 467--81.

Cavagna, G. A., Saibene, F. P. & Margaria, R. 1964. Mechanical work in running. Journal of Applied Physiology 19 (2), 249-256.

Crowninshield, R. D. & Pope, M. H. 1976. The strength and failure characteristics of rat medial collateral ligaments. The Journal of Trauma 16 (2), 99-105.

Cutts, A. 1989. Sarcomere length changes in muscles of the human thigh during walking. Journal of Anatomy 166, 77-84.

Danto, M. I. & Woo, S. L. 1993. The mechanical properties of skeletally mature rabbit anterior cruciate ligament and patellar tendon over a range of strain rates. Journal of Orthopaedic Research 11 (1), 58-67.

Edman, K. A. 1988. Double-hyperbolic force-velocity relation in frog muscle fibres. The Journal of Physiology 404, 301-321.

Eliasson, P., Fahlgren, A., Pasternak, B. & Aspenberg, P. 2007. Unloaded rat Achilles tendons continue to grow, but lose viscoelasticity. Journal of Applied Physiology 103 (2), 459-463.

Farris, D. J. & Sawicki, G. S. 2012. Human medial gastrocnemius force–velocity behavior shifts with locomotion speed and gait. Proceedings of the National Academy of Sciences of the United States of America 109 (3), 977-82.

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

Farris, D., Trewartha, G. & McGuigan, M. 2011. The effects of a 30-min run on the mechanics of the human Achilles tendon. European Journal of Applied Physiology 112 (2), 653-660.

Finni, T., Hodgson, J. A., Lai, A. M., Edgerton, V. R. & Sinha, S. 2006. Muscle synergism during isometric plantarflexion in achilles tendon rupture

patients and in normal subjects revealed by velocity-encoded cine phase-contrast MRI. Clinical Biomechanics 21 (1), 67-74.

Finni, T., Hodgson, J. A., Lai, A. M., Edgerton, V. R. & Sinha, S. 2003. Nonuniform strain of human soleus aponeurosis-tendon complex during submaximal voluntary contractions in vivo. Journal of Applied Physiology 95 (2), 829-837.

Finni, T., Komi, P. V. & Lepola, V. 2000. In vivo human triceps surae and quadriceps femoris muscle function in a squat jump and counter movement jump. European Journal of Applied Physiology 83 (4), 416-426.

Finni, T., Peltonen, J., Stenroth, L. & Cronin, N. J. 2012. On the hysteresis in the human Achilles tendon. Journal of Applied Physiology 114 (4), 515-517.

Foure, A., Nordez, A. & Cornu, C. 2010. Plyometric training effects on Achilles tendon stiffness and dissipative properties. Journal of Applied Physiology 109 (3), 849-854.

Fukashiro, S., Komi, P. V., Järvinen, M. & Miyashita, M. 1995. In vivo Achilles tendon loading during jumping in humans. European Journal of Applied Physiology 71 (5), 453-458.

Fukunaga, T., Kubo, K., Kawakami, Y., Fukashiro, S., Kanehisa, H. & Maganaris, C. N. 2001. In vivo behaviour of human muscle tendon during walking. Proceedings of the Royal Society of London. Series B: Biological sciences 268 (1464), 229-233.

Fung, Y. C. 1993. Biomechanics: Mechanical Properties if Living Tissues. (2nd Edition edition) 175 Fifth Avenue, New York, NY 10010, USA: Springer-Verlag New York, Inc.

Fung, D. T., Wang, V. M., Laudier, D. M., Shine, J. H., Basta-Pljakic, J., Jepsen, K. J., Schaffler, M. B. & Flatow, E. L. 2009. Subrupture tendon fatigue damage. Journal of Orthopaedic Research 27 (2), 264-273.

Galloway, M. T., Jokl, P. & Dayton, O. W. 1992. Achilles tendon overuse injuries. Clinics in Sports Medicine 11 (4), 771-782.

Gerus, P., Rao, G. & Berton, E. 2011. A method to characterize in vivo tendon force–strain relationship by combining ultrasonography, motion capture and loading rates. Journal of Biomechanics 44 (12), 2333-36.

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

Hansen, P., Bojsen-Moller, J., Aagaard, P., Kjaer, M. & Magnusson, S. P. 2006. Mechanical properties of the human patellar tendon, in vivo. Clinical Biomechanics 21 (1), 54-58.

Hausswirth, C., Bigard, A. X. & Guezennec, C. Y. 1997. Relationships between running mechanics and energy cost of running at the end of a triathlon and a marathon. International Journal of Sports Medicine 18 (5), 330-339.

Heglund, N. C. & Taylor, C. R. 1988. Speed, stride frequency and energy cost per stride: how do they change with body size and gait? The Journal of Experimental Biology 138, 301-318.

Herrick, W. C., Kingsbury, H. B. & Lou, D. Y. 1978. A study of the normal range of strain, strain rate, and stiffness of tendon. Journal of Biomedical Materials Research 12 (6), 877-894.

IT'IS Foundation 2013. Tissue properties database. Available in: http://www.itis.ethz.ch/itis-for-health/tissue-properties/database/heat-capacity/.

Järvinen, M., Jozsa, L., Kannus, P., Järvinen, T., Kvist, M. & Leadbetter, W. 1997. Histopathological findings in chronic tendon disorders. Scandinavian Journal of Medicine & Science in Sports 7 (2), 86-95.

Jozsa, L., Kvist, M., Balint, B. J., Reffy, A., Jarvinen, M., Lehto, M. & Barzo, M. 1989. The role of recreational sport activity in Achilles tendon rupture. A clinical, pathoanatomical, and sociological study of 292 cases. The American Journal of Sports Medicine 17 (3), 338-343.

Kannus, P. & Józsa, L. 1997. Human tendons. IL, United States: Human Kinetics.

Kannus, P. & Jozsa, L. 1991. Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. Journal of Bone Joint Surgery 73 (10), 1507-1525.

Ker, R., Alexander, R. & Bennett, M. 1988. Why are mammalian tendons so thick? Journal of Zoology 216 (2), 309-324.

Ker, R. F. 1981. Dynamic tensile properties of the plantaris tendon of sheep (Ovis aries). Journal of Experimental Biology 93 (1), 283-302.

Ker, R. F., Bennett, M. B., Bibby, S. R., Kester, R. C. & Alexander, R. M. 1987. The Spring in the Arch of the Human Foot. Nature 325 (6100), 147-149.

Ker, R., Wang, X. & Pike, A. 2000. Fatigue quality of mammalian tendons. Journal of Experimental Biology 203 (8), 1317-1327.

Komi, P. V., Fukashiro, S. & Järvinen, M. 1992. Biomechanical loading of Achilles tendon during normal locomotion. Clinics in Sports Medicine 11 (3), 521-521-531.

Kongsgaard, M., Aagaard, P., Kjaer, M. & Magnusson, S. P. 2005. Structural Achilles tendon properties in athletes subjected to different exercise modes and in Achilles tendon rupture patients. Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology 99 (5), 1965-1971.

Kubo, K., Kanehisa, H., Kawakami, Y. & Fukunaga, T. 2001a. Effects of repeated muscle contractions on the tendon structures in humans. European Journal of Applied Physiology 84 (1), 162-166.

Kubo, K., Kanehisa, H. & Fukunaga, T. 2002. Effect of stretching training on the viscoelastic properties of human tendon structures in vivo. Journal of Applied Physiology 92 (2), 595-601.

Kubo, K., Kawakami, Y., Kanehisa, H. & Fukunaga, T. 2002. Measurement of viscoelastic properties of tendon structures in vivo. Scandinavian Journal of Medicine & Science in Sports 12 (1), 3-8.

Kubo, K., Kanehisa, H. & Fukunaga, T. 2005. Influences of Repetitive Drop Jump and Isometric Leg Press Exercises on Tendon Properties in Knee Extensors. Journal of Strength & Conditioning Research 19 (4), 864-870.

Kubo, K., Kanehisa, H., Kawakami, Y. & Fukunaga, T. 2001b. Influences of repetitive muscle contractions with different modes on tendon elasticity in vivo. Journal of Applied Physiology 91 (1), 277-282.

Kujala, U. M., Sarna, S. & Kaprio, J. 2005. Cumulative incidence of achilles tendon rupture and tendinopathy in male former elite athletes. Clinical Journal of Sport Medicine : Official Journal of the Canadian Academy of Sport Medicine 15 (3), 133-135.

Kyrolainen, H., Pullinen, T., Candau, R., Avela, J., Huttunen, P. & Komi, P. V. 2000. Effects of marathon running on running economy and kinematics. European Journal of Applied Physiology 82 (4), 297-304.

Leppilahti, J. & Orava, S. 1998. Total Achilles tendon rupture. A review. Sports Medicine 25 (2), 79-100.

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

Lichtwark, G. A., Cresswell, A. G. & Newsham-West, R. J. 2013. Effects of running on human Achilles tendon length-tension properties in the free and gastrocnemius components. The Journal of Experimental Biology 216 (Pt 23), 4388-4394.

Lichtwark, G. A. & Wilson, A. M. 2005. In vivo mechanical properties of the human Achilles tendon during one-legged hopping. Journal of Experimental Biology 208 (24), 4715-4725.

Lieberman, D. E. & Bramble, D. M. 2007. The Evolution of Marathon Running: Capabilities in Humans. Sports Medicine 37 (4-5), 288-290.

Mabuchi, K., Hayatsu, K. & Fujie, H. 1991. Stiffness of canine stifle joint ligaments at relatively high rates of elongation. Journal of Biomechanical Engineering 113 (4), 404-409.

Mademli, L., Arampatzis, A. & Walsh, M. 2008. Age-related effect of static and cyclic loadings on the strain-force curve of the vastus lateralis tendon and aponeurosis. Journal of Experimental Biology 130, 011007.

Mademli, L., Arampatzis, A. & Walsh, M. 2006. Effect of muscle fatigue on the compliance of the gastrocnemius medialis tendon and aponeurosis. Journal of Biomechanics 39 (3), 426-434.

Mademli, L. & Arampatzis, A. 2008. Mechanical and morphological properties of the triceps surae muscle–tendon unit in old and young adults and their interaction with a submaximal fatiguing contraction. Journal of Electromyography and Kinesiology, 18 (1), 89-98.

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

Maganaris, C. N. 2003b. Tendon conditioning: artefact or property? Proceedings of Biological Sciences 270, 39-42.

Maganaris, C. N., Baltzopoulos, V. & Sargeant, A. J. 2000. In vivo measurement-based estimations of the human Achilles tendon moment arm. European Journal of Applied Physiology 83 (4), 363-369.

Maganaris, C. N. & Paul, J. P. 2002. Tensile properties of the in vivo human gastrocnemius tendon. Journal of Biomechanics 35 (12), 1639-1646.

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

Malvankar, S. & Khan, W. S. 2011. Evolution of the Achilles tendon: The athlete's Achilles heel? The Foot 21 (4), 197.

McMahon, T. 1985. The role of compliance in mammalian running gaits. Journal of Experimental Biology 115 (1), 263-282.

Narici, M. V. & Maganaris, C. N. 2006. Adaptability of elderly human muscles and tendons to increased loading. Journal of Anatomy 208 (4), 433-443.

Ng, B. H., Chou, S. M., Lim, B. H. & Chong, A. 2004. Strain rate effect on the failure properties of tendons. Proceedings of the Institution of Mechanical Engineers.Part H, Journal of Engineering in Medicine 218 (3), 203-206.

Noyes, F. R., Delucas, J. L. & Torvik, P. J. 1974. Biomechanics of Anterior Cruciate Ligament Failure: An Analysis of Strain-Rate Sensitivity and Mechanism of Failure in Primates. Journal of Bone and Joint Surgery 56, 236-253.

Pearson, S. J., Burgess, K. & Onambele G. N. L. 2007. Creep and the in vivo assessment of human patellar tendon mechanical properties. Clinical Biomechanics 22 (6), 712-17.

Peltonen, J., Cronin, N. J., Stenroth, L., Finni, T. & Avela, J. 2012. Achilles tendon stiffness is unchanged one hour after a marathon. The Journal of Experimental Biology 215 (20), 3665-3671.

Pike, A., Ker, R. & Alexander, R. 2000. The development of fatigue quality in high- and low-stressed tendons of sheep (Ovis aries). Journal of Experimental Biology 203 (14), 2187-2193.

Pioletti, D. P., Rakotomanana, L. R., Benvenuti, J. & Leyvraz, P. 1998. Viscoelastic constitutive law in large deformations: application to human knee ligaments and tendons. Journal of Biomechanics 31 (8), 753-57.

Pollock, C. M. & Shadwick, R. E. 1994. Relationship between body mass and biomechanical properties of limb tendons in adult mammals. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 266 (3), 1016-21.

Rassier, D. E., MacIntosh, B. R. & Herzog, W. 1999. Length dependence of active force production in skeletal muscle. Journal of Applied Physiology 86 (5), 1445-1457.

Reeves, N. D. 2006. Adaptation of the tendon to mechanical usage. Journal of Musculoskeletal & Neuronal Interactions 6 (6), 174-180.

Reeves, N. D., Maganaris, C. N., Ferretti, G. & Narici, M. V. 2005. Influence of 90-day simulated microgravity on human tendon mechanical properties and the effect of resistive countermeasures. Journal of Applied Physiology 98 (6), 2278-2286.

Riemersma, D. J. & Schamhardt, H. C. 1985. In vitro mechanical properties of equine tendons in relation to cross-sectional area and collagen content. Research in Veterinary Science 39 (3), 263-270.

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

Rosager, S., Aagaard, P., Dyhre-Poulsen, P., Neergaard, K., Kjaer, M. & Magnusson, S. P. 2002. Load-displacement properties of the human triceps surae aponeurosis and tendon in runners and non-runners. Scandinavian Journal of Medicine & Science in Sports 12, 90-98.

Rugg, S. G., Gregor, R. J., Mandelbaum, B. R. & Chiu, L. 1990. In vivo moment arm calculations at the ankle using magnetic resonance imaging (MRI). Journal of Biomechanics 23 (5), 495-497.

Sanjeevi, R. 1982. A viscoelastic model for the mechanical properties of biological materials. Journal of Biomechanics 15 (2), 107-109.

Schechtman, H. & Bader, D. L. 2002. Fatigue damage of human tendons. Journal of Biomechanics, 35 (3), 347-353.

Schechtman, H. & Bader, D. L. 1997. In vitro fatigue of human tendons. Journal of Biomechanics 30 (8), 829-835.

Schechtman, H. & Bader, D. L. 1994. Dynamic characterization of human tendons. Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine 208 (4), 241-248.

Shadwick, R. E. 1990. Elastic energy storage in tendons: mechanical differences related to function and age. Journal of Applied Physiology 68 (3), 1033-1040.

Smith, N. P., Barclay, C. J. & Loiselle, D. S. 2005. The efficiency of muscle contraction. Progress in Biophysics and Molecular Biology 88 (1), 1-58.

Stenroth, L., Peltonen, J., Cronin, N. J., 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), 1537-44.

Ullrich, A. C., Mademli, L. & Arampatzis, A. 2009. Effects of submaximal and maximal long-lasting contractions on the compliance of vastus lateralis tendon and aponeurosis in vivo. Journal of Electromyography and Kinesiology, 19, 476-483.

Vainionpää, M., Tienhaara, E., Raekallio, M., Junnila, J., Snellman, M. & Vainio, O. 2012. Thermographic Imaging of the Superficial Temperature in Racing Greyhounds before and after the Race. The Scientific World Journal 2012.

Viidik, A. 1973. Functional properties of collagenous tissues. International Review of Connective Tissue Research 6, 127-215.

Wang, J. H. 2006. Mechanobiology of tendon. Journal of Biomechanics 39 (9), 1563-1582.

Wang, X.,T., Ker, R.,F. & Alexander, R.,M. 1995. Fatigue rupture of wallaby tail tendons. Journal of Experimental Biology 198 (3), 847-852.

Wang, X. & Ker, R. 1995. Creep rupture of wallaby tail tendons. Journal of Experimental Biology 198 (3), 831-845.

Ward, S. R., Eng, C. M., Smallwood, L. H. & Lieber, R. L. 2009. Are current measurements of lower extremity muscle architecture accurate? Clinical Orthopaedics and Related Research 467 (4), 1074-1082.

Waugh, C. M., Blazevich, A. J., Fath, F. & Korff, T. 2011. Age-related changes in mechanical properties of the Achilles tendon. Journal of Anatomy 220 (2), 144-155.

Wells, J. B. 1965. Comparison of mechanical properties between slow and fast mammalian muscles. The Journal of Physiology 178 (2), 252-269.

Westh, E., Kongsgaard, M., Bojsen-Moller, J., Aagaard, P., Hansen, M., Kjaer, M. & Magnusson, S. P. 2008. Effect of habitual exercise on the structural and mechanical properties of human tendon, in vivo, in men and women. Scandinavian Journal of Medicine & Science in Sports 18 (1), 23-30.

Wilson, A. M. & Goodship, A. E. 1994. Exercise-induced hyperthermia as a possible mechanism for tendon degeneration. Journal of Biomechanics 27 (7), 899-905.

Woo, S. L., Peterson, R. H., Ohland, K. J., Sites, T. J. & Danto, M. I. 1990. The effects of strain rate on the properties of the medial collateral ligament in skeletally immature and mature rabbits: A biomechanical and histological study. Journal of Orthopaedic Research 8 (5), 712-721.

Wren, T. A. L., Lindsey, D. P., Beaupré, G. S. & Carter, D. R. 2003. Effects of Creep and Cyclic Loading on the Mechanical Properties and Failure of Human Achilles Tendons. Annals of Biomedical Engineering 31 (6), 710-717.

Wren, T. A. L., Yerby, S. A., Beaupré, G. S. & Carter, D. R. 2001a. Influence of bone mineral density, age, and strain rate on the failure mode of human Achilles tendons. Clinical Biomechanics 16 (6), 529-534.

Wren, T. A. L., Yerby, S. A., Beaupré, G. S. & Carter, D. R. 2001b. Mechanical properties of the human Achilles tendon. Clinical Biomechanics, 16 (3), 245-251.

ORIGINAL PAPERS

I

IN VIVO MECHANICAL RESPONSE OF HUMAN ACHILLES TENDON TO SINGLE BOUT OF HOPPING EXERCISE.

By

Peltonen J, Cronin NJ, Avela J, Finni T, 2010

Journal of Experimental Biology. 213 (8): 1259–65.

II

ACHILLES TENDON STIFFNESS IS UNCHANGED ONE HOUR AFTER A MARATHON

By

Peltonen J, Cronin N, Stenroth L, Finni T, Avela J, 2012

Journal of Experimental Biology 215(20): 3665–71.

III

VISCOELASTIC PROPERTIES OF THE ACHILLES TENDON IN VIVO

By

Peltonen J, Cronin NJ, Stenroth L, Finni T, Avela J, 2013

Springerplus 2: 212.

Peltonen *et al. SpringerPlus* 2013, **2**:212
http://www.springerplus.com/content/2/1/212

SpringerPlus
a SpringerOpen Journal

RESEARCH Open Access

Viscoelastic properties of the Achilles tendon in vivo

Jussi Peltonen*, Neil J Cronin, Lauri Stenroth, Taija Finni and Janne Avela

Abstract

It has been postulated that human tendons are viscoelastic and their mechanical properties time-dependent. Although Achilles tendon (AT) mechanics are widely reported, there is no consensus about AT viscoelastic properties such as loading rate dependency or hysteresis, in vivo. AT force-elongation characteristics were determined from 14 subjects in an ankle dynamometer at different loading rates using motion capture assisted ultrasonography. AT stiffness and elongation were determined between 10 – 80% of maximum voluntary contraction (MVC) force at fast and slow loading rates. As subjects were unable to consistently match the target unloading rate in the slow condition, AT hysteresis was only calculated for the fast rate. There was a significant difference between the fast and the slow loading rates: 120 ± 6 vs. 21 ± 1% of MVC s^{-1} (mean ± standard error), respectively. However, neither stiffness (193 ± 18 N mm^{-1} vs. 207 ± 22 N mm^{-1}) nor elongation at any force level (13.0 ± 1.2 mm vs. 14.3 ± 0.9 mm at 80% of MVC) were significantly different between the fast and slow loading rates. Tendon hysteresis at the fast rate was 5 ± 2%. As stiffness was not sensitive to loading rate and hysteresis was small, it was concluded that elastic properties prevail over viscous properties in the human AT. The current results support the idea that AT stiffness is independent of loading rate.

Keywords: Stiffness, Hysteresis, Ultrasonography, Loading rate dependency, Medial gastrocnemius

Introduction

Tendon stiffness is the most frequently used parameter to study tendon adaptation due to maturation (Waugh et al. 2011), aging (Narici and Maganaris. 2006), training (Foure et al. 2010; Westh et al. 2008), inactivity (Reeves et al. 2005) and disease (Zhao et al. 2009). However, it has been postulated that human tendons are viscoelastic and that their mechanical properties, like stiffness, depend on the rate at which the load is applied (Fung. 1993; Pioletti et al. 1998; Sanjeevi. 1982). This study was motivated by the observation that in the published literature, there is currently no consensus about the loading rate dependency or hysteresis of human tendons *in vivo*. In addition, viscoelastic behaviour is often disregarded in tendon adaptation studies and mechanical properties are determined at a variety of different loading rates.

In tendons that undergo substantial length changes during movement, like the Achilles tendon (AT), stiffness does not only determine functional properties of the tendon, but also the operational range of its muscle fibers (Fukunaga et al. 2001). Thus, stiffness is an important parameter that influences the whole muscle-tendon unit function. Although the idea of loading rate dependent stiffness has been experimentally refuted in vitro in both human (Wren et al. 2001b) and animal (Ker. 1981; Wang et al. 1995) tendons, it has been recently supported in vivo both in the AT (Gerus et al. 2011) and the patella tendon (Pearson et al. 2007).

Low hysteresis is advantageous for most tendons, because they, unlike muscles, store considerable amounts of elastic energy, which can be utilised in propulsion (Alexander and Bennet-Clark. 1977). While both in vitro and in vivo studies agree that hysteresis is an evident property of tendons, there is no agreement about the amount of hysteresis. In vitro, tendon hysteresis has been shown to be small, only 5–10% (Eliasson et al. 2007; Ker. 1981; Riemersma and Schamhardt. 1985; Wang et al. 1995). In vivo, tendon hysteresis was only shown to be equally small (7%) in the portion of the AT that attaches to soleus muscle (Zhao et al. 2009), but much higher (24%) in the medial gastrocnemius (MG) (Farris et al.

* Correspondence: jussi.t.peltonen@jyu.fi
Department of Biology of Physical Activity, Neuromuscular Research Center, University of Jyväskylä, Jyväskylä, Finland

2011b; Wang et al. 2012) and lateral gastrocnemius (Lichtwark and Wilson. 2005) tendon.

Stiffness can be determined from any tendon, but the AT is perhaps the most frequently explored due to its importance in human locomotion and easy accessibility with ultrasound (US) due to its superficiality and pennated muscle fibres. In the current study, the AT was loaded in a controlled laboratory environment to accurately quantify its viscoelastic properties. The current results are particularly useful to interpret the results of AT adaptation studies; are changes in AT stiffness due to adaptation or due to a variety of different loading rates? The current study will also elucidate the relationship between elastic and viscous properties of the human AT. The study examined the following two research questions:

1. Do AT stiffness and elongation in vivo depend on the rate at which the load is applied?
2. Is AT hysteresis in vivo within the 5 to 10% range that has been commonly reported in vitro?

Materials and methods

Subjects

14 subjects (10 males and 4 females) participated in the study. Their age, height and mass were 36 ± 13 years, 173 ± 11 cm and 67 ± 11 kg (mean ± standard deviation), respectively. All subjects had a life-long training background in physical activities such as ball games and endurance running. Participants were informed about the procedures, benefits and possible risks involved in the study, and they all signed a written consent prior to the study. All methods were approved by the local ethical committee and the study conformed to the standards set by the latest revision of the Declaration of Helsinki.

Protocol

Tendon mechanical properties were assessed in an ankle dynamometer where subjects voluntarily contracted and relaxed their calf muscles to stretch and release the AT. Contractions were performed at two distinctive speeds – fast and slow – to impose the AT to loading rates that are typically used in tendon adaptation studies. Prior to measurements, maximum voluntary contraction (MVC) force was determined as the best out of three trials. Then each subject performed 10 warm-up/practise contractions to stabilise the AT (Maganaris. 2003) and to learn the following characteristics of the trials: target force, which was 80% of MVC for both contractions speeds; and the target duration, which was either 1or 7 seconds for fast and slow contractions, respectively. Duration included both stretching and releasing of the tendon. Subjects received visual feedback of their force production through the monitor in front of them to help them to reach the target force level and durations. Six trials were recorded consisting of three trials for each speed. Trial order was randomised and a one-minute resting period was held between contractions to prevent muscle fatigue. Despite several attempts, subjects were not consistently able to control the release phase of the slow contraction. As a result, only the loading phase of the slow contraction is reported.

Apparatus and collection of data

Tendon mechanical testing was performed in a custom-built ankle dynamometer (University of Jyväskylä, Finland). Subjects were seated with their knee extended, the ankle at a right angle (sole of the foot perpendicular to the shank) and the hip flexed to 60° (Figure 1A). Their right leg was tightly anchored between the back rest and the foot pedal where a force transducer (Precision TB5-C1, Raute, Nastola, Finland) was installed. Possible heel movement was quantified with a potentiometer placed under the heel. Foot reaction force and the heel position were collected with a 16-bit AD-board (CED 1401, Cambridge Electronic Design, England) at 1 kHz and stored on computer for later analysis.

AT length was measured using motion capture assisted ultrasonography (US). With this method, US image coordinates can be transformed to the laboratory coordinate system (CS) for accurate calculation of AT length. To identify the myotendinous junction (MTJ) of the medial gastrocnemius (MG) muscle, a linear array US probe (Aloka 5712, Osaka, Japan) was placed in the sagittal plane over the right leg 2 cm medial to the junction separating the medial and lateral portions of the gastrocnemius muscle. An impedance-matched acoustic pad was placed under the probe to ease propagation of ultrasound waves, and the probe was secured to the leg with elastic bandages (Figure 1B). A digital camera (InLine 250, Fastec Imaging, San Diego, USA) was placed on the ankle dynamometer's left side to image movement of the probe during measurements. The camera's optical axis was perpendicular to the sagittal plane and the camera was focused on the US probe's four reflective markers that were placed on the probe handle to enable movement tracking. The camera and the US unit operated at 125 frames s^{-1} for the fast loading rate and 60 frames s^{-1} for the slow loading rate. The data collection rate was reduced for the slow condition due to memory limitation in the US imaging unit (Aloka Alpha 10, Osaka, Japan). Because the loading rate was several times slower at the slow rate, the lower sampling frequency was also adequate. US images were stored to the imaging unit and the digital camera's video was recorded to computer for later export. The camera and US images were synchronised with a square-wave pulse that was fed into the US unit's digital input and used to trigger a flashing light signal visible on the video.

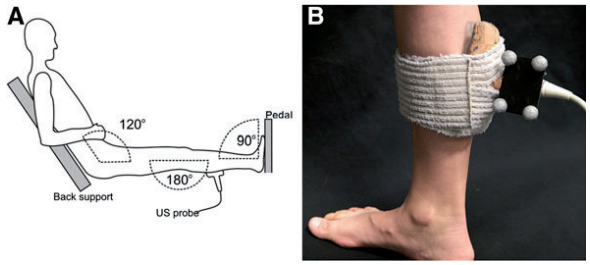

Figure 1 A) Illustration of the ankle dynamometer and B) an ultrasound probe attached on the right leg. A) Reproduced with permission from the Journal of Experimental Biology (Peltonen et al. 2010; doi:10.1242/jeb.033514). **B**) Reflective markers were placed on the ultrasound probe handle to enable motion analysis. The probe was attached in the sagittal plane over the myotendinous junction of the medial gastrocnemius muscle.

Analysis and statistics

AT force was calculated by multiplying the pedal reaction force with a gear ratio (foot lever arm divided by tendon lever arm). Foot lever arm was determined as the distance between the first metatarsal and the centre of the medial malleolus and tendon lever arm as the distance between the medial malleolus and the posterior surface of the calcaneus (Peltonen et al. 2010, 2012). Lever arm lengths were taken as a projection along the sole of the foot when it was perpendicular to the shank. Therefore, lever arms were considered to be perpendicular to the line of force.

AT length was calculated as the distance between the MTJ of the MG muscle and the bony superior and posterior surface of the heel. Initial position of the heel was taken when the foot was properly installed to the pedal. Possible heel displacement in the superior-inferior direction during subsequent muscle contraction was measured with a position sensor located under the heel. MTJ displacement was analysed from the US images with software that exploits pyramidal implementation of the Lukas-Kanade feature tracking (Bouguet, 2000, May). The software requires the user to place nine tracking points over the area of interest as shown in Figure 2. The tracking points were placed just superior to the MTJ along the aponeurosis separating MG and soleus, but still on the side of MG. We have found this placement to yield the most repeatable results. The tracking algorithm has been previously shown to be accurate and repeatable (Magnusson et al. 2003). After tracking, MTJ coordinates of each trial were transformed from the US probe CS to the laboratory CS, which was determined by a stationary video calibration object. The moving CS of the US probe was defined by the four reflective markers placed on the probe handle (Figure 1B). The origin of the US image relative to the probe markers was determined by placing an echogenic marker on the image origin and measuring its distance from the markers. Two dimensional analysis was considered adequate because all movement could be restricted to the sagittal plane (Peltonen et al. 2010). A similar procedure has also been used in space (Gerus et al. 2011; Lichtwark and Wilson. 2005). Tendon elongation was calculated by subtracting initial tendon length at the onset of force production, and strain was calculated by dividing elongation by initial length.

AT force-elongation curves were normalised in time and then averaged resulting in one curve per subject per loading rate. The mean coefficients of variation between each trial for the same subject and the same loading rate were 6% and 7% for the durations of the fast loading and unloading phase, respectively, and 9% for the duration of the slow loading phase. All 14 subjects were included in the analysis of the fast contraction and 10 in the analysis of the slow contraction. Reasons for exclusion were: data were corrupted (1 case); duration of the loading phase deviated by more than 40% of the target duration (1 case); failure to reach the target force level (2 cases).

AT stiffness was calculated as the slope of the least-squares line of the ascending limb of the force-elongation curve between 10% and 80% of MVC force. Loading and

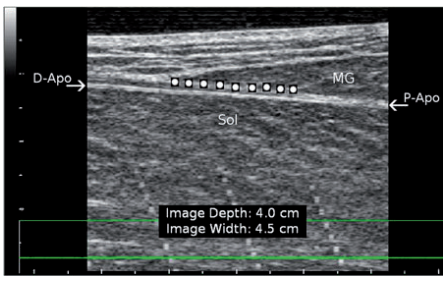

Figure 2 Placement of the tracking markers over the region of interest in the ultrasound image. Nine markers were placed on medial gastrocnemius (MG) muscle along the aponeurosis separating the soleus (Sol) and MG muscles. D-Apo = distal aponeurosis; P-Apo = proximal aponeurosis.

strain rates were calculated as the slope of the force-time and strain-time curves, respectively. Hysteresis was calculated by subtracting the area under the descending limb of the force-elongation curve from the area under the ascending limb and dividing the difference by the area under the ascending limb (Figure 3).

Loading phases of individual force-elongation curves were further averaged to yield separate loading curves for the slow and fast contractions (Figure 4). Averaging was done at 10% intervals ranging from 10% to 80% of MVC and included only those subjects that had data for both loading rates (N = 10).

Wilcoxon's signed-rank test was used to test differences in stiffness and elongation between the fast and slow contractions. Linearity of average force elongation curves was tested with a least squares linear regression method. The level of significance was always set to $P < 0.05$. Errors in figures and tables are the standard error (SE) of the mean.

Results

Table 1 displays AT loading rate sensitivity and hysteresis. The average fast loading rate was 120 ± 6% of MVC s^{-1} (mean ± SE) and it was significantly higher than the average slow loading rate, 21 ± 1% of MVC s^{-1} (N = 10, $P < 0.05$). However, AT stiffness did not change between the fast and slow loading rates being 193 ± 22 N mm^{-1} and 207 ± 18 N mm^{-1}, respectively (N = 10, P = 0.105).

Tendon hysteresis was determined for all subjects at the fast rate (Table 1). Hysteresis was on average 5 ± 2% (N = 14) and it ranged from −7% to 21%. There was no difference between the mean loading time (0.589 s) and the mean unloading time (0.588 s) at the fast rate (N = 14, P = 0.890). The possible relationship between hysteresis and the difference in loading and unloading time was also tested, but the correlation coefficient proved to be non-significant (N = 14, R = −0,425, P = 0.129). It must be noted here that negative hysteresis means that the tendon released more energy than it originally stored, which is not possible. This issue is addressed further in the discussion.

Figure 4 shows average AT elongations at 10% intervals of MVC. At the maximum measured force level, which was 80% of MVC, the AT elongation was 14.3 ± 1.2 mm at the fast rate and 13.0 ± 0.9 mm at the slow rate, but the difference was not statistically significant (N = 10, P = 0.064). The same was true for all force levels: there were no statistical differences in AT elongation.

Discussion

The major findings of the current study were that: 1) AT stiffness and elongation were independent of the loading rate and 2) AT hysteresis was small (5% on average). The current data are in line with the majority of previous in vitro tendon studies indicating time-independent mechanical behaviour (Abrahams. 1967; Ker. 1981; Mabuchi et al. 1991; Noyes et al. 1974; Wang et al. 1995; Woo et al. 1990; Wren et al. 2001b) and small hysteresis (Bennett et al. 1986; Eliasson et al. 2007; Ker. 1981; Riemersma and Schamhardt. 1985; Wang et al. 1995).

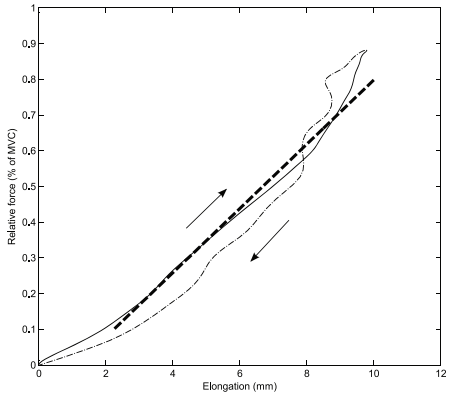

Figure 3 Example of tendon stiffness and hysteresis deduction from the load-deformation curve. Stiffness (broken thick line) was calculated as the slope of the ascending limb (solid line) of the force-elongation curve between 10-80% of MVC. Hysteresis was calculated as the area between the ascending (solid line) and descending limb (dash-dot line). MVC = maximum voluntary contraction.

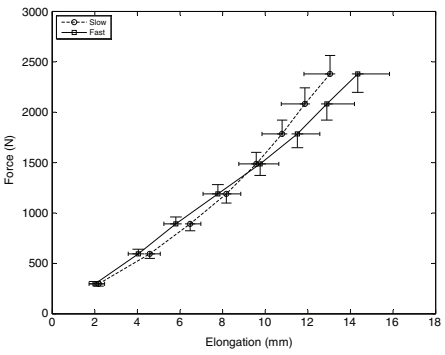

Figure 4 Average Achilles tendon force-elongation curves at the fast and slow loading rates. The data points are given at 10% intervals ranging from 10–80% of maximum voluntary force. The solid line and square markers indicate the fast rate, and the broken line and circle markers indicate the slow rate. Elongation was not significantly different between the fast and slow rates at any force level.

Table 1 Achilles tendon loading rate sensitivity and hysteresis

		Loading rate (% of MVC s^{-1})		Stiffness (N mm^{-1})		Hysteresis (%)
Subject	Sex	Slow	Fast	Slow	Fast	Fast
1	M	18	91	300	267	-7
2	M	20	151	255	310	15
3	M	20	126	191	168	-1
4	F	20	108	196	179	-4
5	F	23	126	273	241	-1
6	M	19	125	156	124	21
7	M	19	97	247	253	6
8	M	23	108	176	154	6
9	F	21	118	144	119	13
10	M	27	153	132	120	-4
11	M		144		227	-4
12	M		180		188	9
13	F		111		132	6
14	M		264		279	13
mean (subjects 1-10)		21	120	207	193	4
SE (subjects 1-10)		1	6	18	22	3
mean (all)			136		197	5
SE (all)			12		17	2

MVC maximum voluntary contraction, *SE* standard error.

Influence of tendon material

Early failure experiments demonstrated that ligaments were more prone to ruptures at high strain rates than at low strain rates (Crowninshield and Pope. 1976; Noyes et al. 1974). Because later studies did not find such a relationship (Danto and Woo. 1993; Ng et al. 2004; Woo et al. 1990; Wren et al. 2001a, 2001b), it has been postulated that tendon viscoelastic properties may vary (Wren et al. 2001b). The finding that high-stressed flexor tendons have a lower hysteresis than low-stressed extensor tendons supports this idea (Shadwick. 1990). Because high-stressed tendons have also been shown to have higher fatigue resistance than low-stressed tendons (Pike et al. 2000), it has been postulated that high-stressed tendons are made of different material, possibly to compensate for a lower safety factor (Ker et al. 2000). In humans, the AT has to withstand stresses around 80 MPa (Lichtwark and Wilson. 2005) and can be categorised as a high-stressed tendon. In comparison, maximum stress in the patella tendon may be only half of that, 40 MPa (Hansen et al. 2006). Thus, functional requirements may explain why the patella tendon and the AT are made of different material and why the patella tendon exhibits loading rate sensitivity (Pearson et al. 2007), whereas the AT in the current study did not.

All subjects in the current study had a history of regularly stressing their AT's in activities that require cyclic stretching and releasing of the AT, which could explain their rather non-viscous properties. The exact mechanism of tendon mechanical adaptation is not understood, but there is substantial evidence showing that tendon stiffness increases during maturation (Waugh et al. 2011) and training (Westh et al. 2008), and decreases during aging (Narici and Maganaris. 2006) and inactivity (Reeves et al. 2005). However, there is usually no clear evidence of causality; for example, an age-related decrease in stiffness may be a consequence of the aging process, but it may also be caused by decreased physical activity and loss of muscle force, therefore suggesting that tendon adapts to usage (Stenroth et al. 2012). Alterations in tendon hysteresis are much less frequently reported than changes in stiffness. However, there is some evidence to suggest that AT hysteresis could decrease after plyometric training (Foure et al. 2010) without any significant change in AT cross-sectional area. This supports the adaptation to usage theory and indicates that by regularly engaging in physical activities, the current subjects may have achieved or maintained an AT that has a small hysteresis and is suitable for efficient elastic energy storage and return.

Implications for human movement

Low hysteresis is advantageous for tendon not only because it enables high elastic energy return but also because it minimises heat damage (Alexander. 2002). This applies to walking, running and jumping, during which high efficiency is required. On the contrary, during landing, where mechanical energy is absorbed rather than

stored and returned, high hysteresis could be useful. This is probably unnecessary because tendon acts as an energy buffer by rapidly stretching and storing energy that is then slowly absorbed during subsequent active muscle lengthening. This substantially lowers energy absorption rate as well as muscle eccentric contraction velocity – as compared to fast muscle stretch – and supposedly protects against muscle damage (Konow et al. 2011). It is difficult to imagine how varying stiffness could improve this ability. Higher stiffness would obviously result in lower tendon strain. However, reducing tendon strain also reduces strain energy storage and may compromise the tendon's ability to act as an energy buffer. Thus, it seems plausible that tendon strains are proportional to the load and independent of the loading rate.

In the current study, the mean slow strain rate was 1.7% s^{-1} and the mean fast strain rate was 10% s^{-1}. Even faster strain rates are achieved in running (15 – 70% s^{-1}) (Farris et al. 2011a; Lichtwark et al. 2007), but the current strain rate range overlaps with that of walking (Lichtwark et al. 2007). The quite substantial strain rate range (6-fold difference), without any noticeable changes in tendon stiffness or elongation, indicates that AT mechanical behaviour may be strain rate independent throughout the physiological strain rate range. Regardless of whether the current results can be generalised to full speed motion or not, they provide evidence that the strain rates that are typically used in AT mechanical testing do not affect its stiffness or elongation.

Methodological considerations

In the published literature, AT elongation has been determined with several methods. The challenge has been to take into account the displacements of the myotendinous junction, the heel and the US probe. Sometimes these parameters have been estimated, but this could lead to overestimation of AT elongation by 40% (Gerus et al. 2011). The current study does not suffer from this limitation. The AT elongation was calculated by measuring both heel movement and MTU displacement within the same contraction, without any corrections, with a method that combines ultrasonography and motion analysis. We consider this method to be the most accurate to date. Because AT force was estimated, it is influenced by variation in moment arm lengths. We have verified with pressure insoles that the point of application of force moves slightly towards the 1st metatarsal as the force increases, thus increasing the foot lever arm length. However, the heel lever arm length increases as well (Rugg et al. 1990) and may counterbalance this effect. Therefore, we have used a constant gear ratio in our calculations.

The current mean force-elongation curves (Figure 4) were rather linear: Pearson's linear correlation coefficients were R = 0.998 and R = 0.999 for the fast and slow loading rates, respectively. Low stiffness, or toe region, was either absent or small, as indicated by the intercept of the linear regression that was not significantly different from zero at the fast rate (student's t-test; P = 0.095) and only slightly different from zero at the slow rate (P = 0.037). The lack of toe region was probably due to the initial tension (approximately 0.3 kN) acting on the AT at the onset of muscle contraction. Due to the initial tension, possible fiber slack is removed and tendon is stretched within the linear region from the beginning of the contraction. This explains why the current average hysteresis is low (5%) and comparable to those achieved during mechanical testing of isolated tendons (Ker. 1981).

In the current study, the range in AT hysteresis (Table 1) was 28% (from −7% to 21%). No single reason for this variation has been yet identified, but it is typical when determining tendon hysteresis in vivo. Previously reported ranges include 43% during running (from 2% to 45%) (Farris et al. 2011b) and 24% during one-legged hopping (from 15% to 39%) (Lichtwark and Wilson. 2005). In viscous material, variations in hysteresis could be induced by alternating loading and unloading rates. However, this idea was refuted in the current study, because there was no difference between the fast loading and unloading times. We have recently suggested that variation in hysteresis can be attributed to signal desynchronisation and differentiating synergist muscle activation (Finni et al. 2012). For example, if the total duration of the loading and unloading phases is 1 second, and the sampling rate is 100 Hz, desynchronisation of 1 frame may under- or overestimate hysteresis by 10% (Finni et al. 2012). There are methods to overcome this problem and signal averaging is one of them: Averaging force-elongation curves over several trials reduces the effect of random desynchronisation by one frame. The more compelling challenge is the anatomy of the tendon, because the AT is a common tendon of the three heads of the triceps surae muscles, and each head can stretch the AT independently causing regional strain variations (Finni et al. 2006). This may cause random and systematic errors. While random errors are treated by averaging, systematic errors are harder to detect. However, their influence on conclusions can be minimized by having the same settings, e.g. joint angles, for both the fast and the slow rate. In summary, measurement uncertainties are an inevitable consequence of determining human AT hysteresis in vivo. As a result, experimentally deduced hysteresis may also include negative values if the mean hysteresis is low (less than 10% as indicated by isolated tendons). Considering negative hysteresis values to be erroneous and rejecting them may lead to biased results (Finni et al. 2012). This could also explain why reported values for hysteresis are typically higher in vivo than in vitro.

Conclusion

In the AT of physically active individuals, elastic properties prevail over viscous properties. Thus, the AT is well suited to elastic energy storage and return during human walking, running and jumping. The current results are in agreement with the majority of in vitro studies that indicate rather non-viscous properties in tendons across species. However, the findings about tendon hysteresis remain inconclusive. In vitro studies typically demonstrate low tendon hysteresis (<10%), whereas in vivo studies have often shown higher hysteresis (>20%). Our results support the former, which we believe represent more physiological values. It is postulated that the differences in hysteresis in living tendons could be attributed to methodological variations as well as material differences in tendons induced by physical activity backgrounds and/or tendon anatomical location and function. The current results support the idea that tendon stiffness is not affected by the rate at which the load is applied during tendon mechanical testing in vivo.

Abbreviations

AT: Achilles tendon; CS: Coordinate system; MG: Medial gastrocnemius; MTJ: Myotendinous junction; MVC: Maximum voluntary contraction; US: Ultrasonography.

Competing interests

The authors have no conflicts of interest to report.

Authors' contributions

TF and JA are the supervisors of JP and they helped to design the study and monitored the process. JP participated in designing the study and collecting the data, analyzed the data and drafted the manuscript. LS and NC participated in collecting the data and in interpretation of the results. NC also helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors gratefully acknowledge the technical staff at the University of Jyväskylä for assistance with this study.

Received: 8 April 2013 Accepted: 25 April 2013
Published: 8 May 2013

References

Abrahams M (1967) Mechanical behaviour of tendonIn vitro. Medical and Biological Engineering and Computing 5:433–443

Alexander RM (2002) Tendon elasticity and muscle function. Comp Biochem Physiol A Mol Integr Physiol 133:1001–1011. doi:10.1016/S1095-6433(02)00143-5

Alexander RM, Bennet-Clark H (1977) Storage of elastic strain energy in muscle and other tissues. Nature 265:114–117

Bennett MB, Ker RF, Imery NJ, Alexander RM (1986) Mechanical properties of various mammalian tendons. J Zool 209:537–548. doi:10.1111/j.1469-7998.1986.tb03609.x

Bouguet JY (2000) Pyramidal Implementation of the Lucas Kanade Feature Tracker. Description of the Algorithm. http://robots.stanford.edu/cs223b04/algo_tracking.pdf. Accessed 30 April 2013

Crowninshield RD, Pope MH (1976) The strength and failure characteristics of rat medial collateral ligaments. J Trauma 16:99–105

Danto MI, Woo SL (1993) The mechanical properties of skeletally mature rabbit anterior cruciate ligament and patellar tendon over a range of strain rates. J Orthop Res 11:58–67

Eliasson P, Fahlgren A, Pasternak B, Aspenberg P (2007) Unloaded rat Achilles tendons continue to grow, but lose viscoelasticity. J Appl Physiol 103:459–463. doi:10.1152/japplphysiol.01333.2006.

Farris D, Trewartha G, McGuigan M (2011a) The effects of a 30-min run on the mechanics of the human Achilles tendon. Eur J Appl Physiol 112:653–660. doi:10.1007/s00421-011-2019-8.

Farris DJ, Trewartha G, Polly McGuigan M (2011b) Could intra-tendinous hyperthermia during running explain chronic injury of the human Achilles tendon? J Biomech 44:822–826

Finni T, Hodgson JA, Lai AM, Edgerton VR, Sinha S (2006) Muscle synergism during isometric plantarflexion in achilles tendon rupture patients and in normal subjects revealed by velocity-encoded cine phase-contrast MRI. Clin Biomech 21:67–74

Finni T, Peltonen J, Stenroth L, Cronin NJ (2012) On the hysteresis in the human Achilles tendon. J Appl Physiol. doi:10.1152/japplphysiol.01005.2012

Foure A, Nordez A, Cornu C (2010) Plyometric training effects on Achilles tendon stiffness and dissipative properties. J Appl Physiol 109:849–854. doi:10.1152/japplphysiol.01150.2009.

Fukunaga T, Kubo K, Kawakami Y, Fukashiro S, Kanehisa H, Maganaris CN (2001) In vivo behaviour of human muscle tendon during walking. Proc Biol Sci 268:229–233. doi:10.1098/rspb.2000.1361.

Fung YC (1993) Biomechanics: Mechanical Properties if Living Tissues. Springer-Verlag New York, Inc., 175 Fifth Avenue, New York, NY 10010, USA

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

Hansen P, Bojsen-Moller J, Aagaard P, Kjaer M, Magnusson SP (2006) Mechanical properties of the human patellar tendon, in vivo. Clin Biomech 21:54–58. doi:10.1016/j.clinbiomech.2005.07.008.

Ker RF (1981) Dynamic tensile properties of the plantaris tendon of sheep (Ovis aries). J Exp Biol 93:283–302

Ker R, Wang X, Pike A (2000) Fatigue quality of mammalian tendons. J Exp Biol 203:1317–1327

Konow N, Azizi E, Roberts TJ (2011) Muscle power attenuation by tendon during energy dissipation. Proc Biol Sci. doi:10.1098/rspb.2011.1435

Lichtwark GA, Wilson AM (2005) In vivo mechanical properties of the human Achilles tendon during one-legged hopping. J Exp Biol 208:4715–4725. doi:10.1242/jeb.01950.

Lichtwark GA, Bougoulias K, Wilson AM (2007) Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. J Biomech 40:157–164. doi:10.1016/j.jbiomech.2005.10.035.

Mabuchi K, Hayatsu K, Fujie H (1991) Stiffness of canine stifle joint ligaments at relatively high rates of elongation. J Biomech Eng 113:404–409

Maganaris CN (2003) Tendon conditioning: artefact or property? Proc Biol Sci 270:39–42

Magnusson SP, Hansen P, Aagaard P et al (2003) Differential strain patterns of the human gastrocnemius aponeurosis and free tendon, in vivo. Acta Physiol Scand 177:185–195

Narici MV, Maganaris CN (2006) Adaptability of elderly human muscles and tendons to increased loading. J Anat 208:433–443. doi:10.1111/j.1469-7580.2006.00548.x.

Ng BH, Chou SM, Lim BH, Chong A (2004) Strain rate effect on the failure properties of tendons. Proc Inst Mech Eng H 218:203–206

Noyes FR, Delucas JL, Torvik PJ (1974) Biomechanics of Anterior Cruciate Ligament Failure: An Analysis of Strain-Rate Sensitivity and Mechanism of Failure in Primates. J Bone Joint Surg Am 56:236–253

Pearson SJ, Burgess K, Gladys N, Onambele L (2007) Creep and the in vivo assessment of human patellar tendon mechanical properties. Clin Biomech (Bristol, Avon) 22:712–717

Peltonen J, Cronin NJ, Avela J, Finni T (2010) In vivo mechanical response of human Achilles tendon to a single bout of hopping exercise. J Exp Biol 213:1259–1265. doi:10.1242/jeb.033514.

Peltonen J, Cronin NJ, Stenroth L, Finni T, Avela J (2012) Achilles tendon stiffness is unchanged one hour after a maraton. J Exp Biol 215:3665–3671. doi:10.1242/jeb.068874.

Pike A, Ker R, Alexander R (2000) The development of fatigue quality in high- and low-stressed tendons of sheep (Ovis aries). J Exp Biol 203:2187–2193

Pioletti DP, Rakotomanana LR, Benvenuti J, Leyvraz P (1998) Viscoelastic constitutive law in large deformations: application to human knee ligaments and tendons. J Biomech 31:753–757

Reeves ND, Maganaris CN, Ferretti G, Narici MV (2005) Influence of 90-day simulated microgravity on human tendon mechanical properties and the effect of resistive countermeasures. J Appl Physiol 98:2278–2286. doi:10.1152/japplphysiol.01266.2004.

Riemersma DJ, Schamhardt HC (1985) In vitro mechanical properties of equine tendons in relation to cross-sectional area and collagen content. Res Vet Sci 39:263–270

Rugg SG, Gregor RJ, Mandelbaum BR, Chiu L (1990) In vivo moment arm calculations at the ankle using magnetic resonance imaging (MRI). J Biomech 23:495–497. doi:10.1016/0021-9290(90)90305-M.

Sanjeevi R (1982) A viscoelastic model for the mechanical properties of biological materials. J Biomech 15:107–109. doi:10.1016/0021-9290(82)90042-2.

Shadwick RE (1990) Elastic energy storage in tendons: mechanical differences related to function and age. J Appl Physiol 68:1033–1040

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. J Appl Physiol. doi:10.1152/japplphysiol.00782.2012

Wang XT, Ker RF, Alexander RM (1995) Fatigue rupture of wallaby tail tendons. J Exp Biol 198:847–852

Wang HK, Lin KH, Su SC, Shih TT, Huang YC (2012) Effects of tendon viscoelasticity in Achilles tendinosis on explosive performance and clinical severity in athletes. Scand J Med Sci Sports 22:147–155. doi:10.1111/j.1600-0838.2012.01511.x.

Waugh CM, Blazevich AJ, Fath F, Korff T (2011) Age-related changes in mechanical properties of the Achilles tendon. J Anat 220:144–155. doi:10.1111/j.1469-7580.2011.01461.x.

Westh E, Kongsgaard M, Bojsen-Moller J et al (2008) Effect of habitual exercise on the structural and mechanical properties of human tendon, in vivo, in men and women. Scand J Med Sci Sports 18:23–30. doi:10.1111/j.1600-0838.2007.00638.x.

Woo SL, Peterson RH, Ohland KJ, Sites TJ, Danto MI (1990) The effects of strain rate on the properties of the medial collateral ligament in skeletally immature and mature rabbits: A biomechanical and histological study. J Orthop Res 8:712–721

Wren TAL, Yerby SA, Beaupré GS, Carter DR (2001a) Mechanical properties of the human Achilles tendon. Clin Biomech 16:245–251

Wren TAL, Yerby SA, Beaupré GS, Carter DR (2001b) Influence of bone mineral density, age, and strain rate on the failure mode of human Achilles tendons. Clin Biomech (Bristol, Avon) 16:529–534

Zhao H, Ren Y, Wu Y, Liu SQ, Zhang L (2009) Ultrasonic evaluations of Achilles tendon mechanical properties poststroke. J Appl Physiol 106:843–849. doi:10.1152/japplphysiol.91212.2008.

doi:10.1186/2193-1801-2-212
Cite this article as: Peltonen *et al.*: Viscoelastic properties of the Achilles tendon in vivo. *SpringerPlus* 2013 **2**:212.

IV

RELATION BETWEEN ACHILLES TENDON HYSTERESIS AND INCREASE OF SKIN TEMPERATURE DURING CYCLIC STRETCHING AND SHORTENING IN VIVO

By

Peltonen J, Cronin NJ, Toivonen J, Finni T, Avela J, 2014

Submitted for publication

Relation between Achilles tendon hysteresis and increase of skin temperature during cyclic stretching and shortening in vivo

Authors: Jussi Peltonen, Johanna Toivonen, Neil J. Cronin, Taija Finni, Janne Avela

Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Finland

Running head: Tendon hysteresis and increase of temperature

Keywords: Infrared thermography, tendon hyperthermia, ultrasonography, dissipated heat, absorbed heat, force-length relation, skin temperature

Word count (abstract/article): 212/3 851

Number of figures/tables: 6/2

Address for correspondence: Jussi Peltonen

Viveca 226, P.O. Box 3, Jyväskylä 40014, Finland

E-mail: jussi.t.peltonen@jyu.fi

Tel: +358-014-260-2090

Fax: +358-014-260-2070

ABSTRACT

The purpose of the current study was to investigate the origin of variation in human Achilles tendon (AT) hysteresis, which has been shown to be relatively high in vivo, from 3% to 35 %, as compared to 7 % hysteresis in excised tendons. It was postulated that if variation in hysteresis is of physiological origin, higher AT hysteresis will be related to higher increase of AT temperature. AT hysteresis was calculated from tendon force-length data (N = 19) obtained during 15 voluntary contractions in an ankle dynamometer that induced stretch to the tendon. AT force was calculated from measured pedal reaction force and AT length was deduced using motion capture assisted ultrasonography. Increase of AT temperature was measured from skin overlaying the tendon with infrared thermography imaging. The results showed that AT hysteresis was 11 ± 10 % (mean ± standard deviation) and corresponding increase of AT skin temperature was 0.025°C ± 0.014°C per stretch-shorten cycle (N = 19; P < 0.001; significantly different from zero). Because AT hysteresis and increase of skin temperature were positively correlated (N = 19, R = 0.471, P = 0.042), it was concluded that individual differences in AT temperature rise may be explained by hysteresis. Whether or not this relation still persists after longer exposure times, during which AT may reach damaging temperature, remains to be elucidated in future studies.

INTRODUCTION

Hysteresis, defined as percentage of elastic strain energy lost to heat during stretch-shorten cycle, increases the temperature of the tendon, to values as high as 45°C in galloping horses (Wilson and Goodship, 1994) and 41°C in the human Achilles tendon (AT) during running (Farris et al., 2011). When excised tendons are tested, they consistently demonstrate hysteresis around 7 % (Bennett et al., 1986;Ker, 1981). By contrast, in vivo testing of the AT has revealed that hysteresis may exceed 20 % (Foure et al., 2010;Kubo et al., 2002;Lichtwark and Wilson, 2005;Maganaris, 2003). In addition to high hysteresis, in vivo measurements also demonstrate high inter-individual variation in AT hysteresis. Thus increase of temperature should also vary individually during cyclic stretching and shortening of the tendon. To our knowledge, no previous study has simultaneously measured AT hysteresis and corresponding increase of AT temperature in vivo.

In the current study, increase of AT temperature was assessed with infrared thermography (IRT) imaging. Thermography cameras detect radiation in the infrared range of the electromagnetic spectrum (roughly 9–14 μm). Because infrared radiation is emitted by all objects above absolute zero, thermography makes it possible to see how an object's temperature changes over time. IRT imaging has previously been used in a wide range of applications from study of animal thermoregulation (Vainionpää et al., 2012) to clinical diagnostics (Arora et al.,

2008). Due to the proximity of the AT to the overlying skin, infrared thermography could potentially also be used to assess AT temperature changes.

The purpose of the current study was to investigate the relation between AT hysteresis and increase of AT skin temperature. A statistical relationship would indicate that variation in AT hysteresis is of physiological origin rather than a measurement artefact. It was postulated that AT hysteresis, derived from tendon force-length relation, would correlate with increase of AT skin temperature, assessed with IRT. Since heat is a form of energy, dissipated heat, the energy that is lost to heat during elastic recoil, was also compared to absorbed heat, which was calculated based on the increase of AT temperature.

MATERIALS AND METHODS

Subjects

19 males volunteered for the study. Their age, height and mass were 27 ± 5 years, 181 ± 6 cm and 78 ± 12 kg (mean ± standard deviation), respectively (Table 1). Participants were informed about the procedures, benefits and possible risks involved in the study, and they all signed a written consent prior to the study. All methods were approved by the local ethical committee and the study conformed to the standards set by the latest revision of the Declaration of Helsinki.

Pilot study on thermal convection

Prior to measurements, a pilot study was conducted to verify that AT skin temperatures are not affected by heat transferred from the proximal muscles. Two warm water bags (2 x 500 grams) were placed over medial and later gastrocnemius to increase temperature of the muscles independently of the tendon. Skin temperatures were assessed from IRT images from three spots: 1) free AT (3 cm proximal to calcaneus); 2) Soleus AT (3 cm proximal to myotendinous junction of Soleus); and 3) gastrocnemius muscles (average from 5 cm and 3 cm proximal to myotendinous junction of medial and lateral gastrocnemius). Temperatures were analysed four times: 1) before exposure to warm water; 2) after exposure to 41°C water bags; 3) after exposure to 46°C water bags; and 4) after 5 minutes recovery. Figure 1 shows the results of these tests for two subjects. Even though gastrocnemius skin temperature increased from 31°C (subject 1) or 32°C (subject 2) to 37°C or 38°C, free AT or Soleus AT temperatures did not increase. Thus it could be assumed in the present study that increase of AT skin temperature is due to heat produced inside the AT itself rather than heat transferred from proximal muscles.

Protocol

AT mechanical testing and infrared thermography were conducted in seated position. Voluntary attempts were made to plantar flex the ankle against a stationary pedal with a force transducer installed inside. During these attempts AT was stretched and shortened. A computer screen in front of the subjects enabled them to control the pace and strength of their

contractions. At the beginning of the test, force was gradually increased towards maximum voluntary contraction (MVC) force, which was determined as the best out of three trials. Target force for all subsequent contractions was then set to 80% of MVC. Data for AT hysteresis and skin temperature change was then collected during total of 15 repeated contractions, divided into three sets of five repetitions (Fig. 3). This division was made due to space limitations in the ultrasound recording unit. A twenty seconds break was held between sets. Contraction frequency was set to 1 Hz. This frequency was chosen according to pilot testing, where it could be comfortably and accurately repeated for several seconds. AT skin temperature was recorded with an infrared camera prior to the 1st submaximal contraction. The recording continued throughout the 15 contractions and follow-up period, with a total recording time of 10 minutes.

Apparatus and collection of data

Free AT length and cross sectional area were assessed prior to the mechanical testing with an ultrasound (US) unit (Aloka Alpha 10, Osaka, Japan) and 4 cm linear array probe (Aloka 5411, Osaka, Japan). As the subject lay prone on a table, the right ankle at 90 degrees, sagittal US scans were taken along the AT to identify the most proximal insertion to calcaneus and the myotendinous junction (MTJ) of soleus muscle. The free AT length was then taken as the distance between the insertion and the soleus MTJ. AT cross-sectional area was obtained from a transverse scan in the mid-tendon.

For AT mechanical testing, subjects were seated in a custom-built ankle dynamometer (University of Jyväskylä, Finland). Their knee was fully extended, the ankle at a right angle (sole of the foot perpendicular to the shank) and the hip flexed to 60°. Their right leg was tightly anchored between the back rest and the foot pedal (Fig. 2) where a force transducer (Precision TB5-C1, Raute, Nastola, Finland) was installed. Possible heel movement was quantified with a potentiometer placed under the heel. Foot reaction force and the heel position were collected with a 16-bit AD-board (CED 1401, Cambridge Electronic Design, England) at 1 kHz and stored on computer for later analysis.

To assess AT length during the contractions, AT length was taken as the length between the heel and the MTJ of the medial gastrocnemius (MG) muscle with the combination of US imaging and motion capture. With this method, MTJ coordinates in the local US reference frame can be transformed to the laboratory reference frame. To identify the MTJ of the MG muscle, a 6 cm linear array probe (Aloka 5712, Osaka, Japan) was placed in the sagittal plane over the right leg 2 cm medial to the junction separating the medial and lateral portions of the gastrocnemius muscle. An impedance-matched acoustic pad was placed under the probe to ease propagation of ultrasound waves, and the probe was secured to the leg with elastic bandages. A digital camera (InLine 250, Fastec Imaging, San Diego, USA) was placed on the ankle dynamometer's left side to image movement of the probe during measurements. The camera's optical axis was perpendicular to the sagittal plane and the camera was focused on the US probe's four reflective

markers that were placed on the probe handle to enable movement tracking (Peltonen et al., 2012). The camera and the US unit operated at 60 frames s^{-1}. US and camera videos were recorded on a hard drive for later export and analysis. The camera and US images were synchronised with a square-wave pulse that was fed into the US unit's digital input and used to trigger a flashing light signal visible on the video.

An infrared camera (FLIR A300, FLIR Systems AB, Danderyd, Sweden) with a focal plane array detector (size: 320 x 240 pixels; spectral range: 7.5 – 13 μm) and thermal sensitivity of < 0.05C° was used to assess AT skin temperature. The camera's lens was placed at a fixed distance of 0.5 m from the subject and it was focused on the posterior surface of the free AT. Camera's recording rate was set to 0.1 Hz. As infrared thermography is limited by its ability to record only surface temperatures, a follow-up period after the last contraction was necessary for heat to conduct from the tendon core to the skin surface.

Calculations and statistics

AT force was calculated by multiplying the pedal reaction force with a gear ratio (foot lever arm divided by tendon lever arm). Foot lever arm was determined as the distance between the first metatarsal and the centre of the medial malleolus and tendon lever arm as the distance between the medial malleolus and the posterior surface of the calcaneus (Peltonen et al., 2012). Lever arm lengths were taken as a projection along the sole of the foot when it was perpendicular to the shank. Therefore, lever arms were considered to be perpendicular to the line of force.

AT length was calculated as the distance between the MTJ of the MG muscle and the bony superior and posterior surface of the heel. Initial position of the heel was taken when the foot was properly installed to the pedal. Possible heel displacement in the superior-inferior direction during subsequent muscle contraction was measured with a position sensor located under the heel. MTJ displacement was analysed from the US images with software that exploits pyramidal implementation of the Lukas-Kanade feature tracking (Bouguet, 2001). The software requires the user to place nine tracking points over the area of interest. The tracking points were placed just superior to the MTJ along the aponeurosis separating MG and soleus, but still on the side of MG. We have found this placement to yield the most repeatable results. The tracking algorithm has been previously shown to be accurate and repeatable (Magnusson et al., 2003). After tracking, MTJ coordinates were transformed from the US reference frame to the laboratory reference frame, determined by a stationary video calibration object. The movement of the US reference frame was defined by the four reflective markers placed on the probe handle (Fig. 2). The origin of the US reference frame was determined by placing an echogenic object on the image origin and measuring its distance from the reflective markers. Two dimensional analysis was considered adequate because all movement could be restricted to the sagittal plane (Peltonen et al., 2012). A similar procedure has also been used in space (Gerus et al., 2011;Lichtwark and Wilson, 2005). Tendon elongation was calculated by subtracting initial tendon length at the onset of force production from tendon length during the contraction.

AT force-elongation curves were obtained by first synchronizing force and elongation data and then separating each contraction by cutting the data at the 5% MVC force level. This can be visualized as a non-zero intercept in Fig. 5. Data exclusion criteria were: 1) contraction duration deviates more than 15 % from mean duration; and 2) peak force level less than 80% of MVC. Data from contractions that were accepted for analysis from a given set were then time normalized and averaged to yield a single force-elongation curve per set. Stored energy was calculated as area under the ascending limb of the force-elongation curve and dissipated heat by subtracting the area under the descending limb from the area under the ascending limb. Hysteresis was calculated by dividing the dissipated heat by the stored energy. The reported hysteresis is the average of analysed curves. To ease comparison between subjects, the initial force applied to the AT when the foot was placed on the pedal was subtracted from all force recordings. Thus, the reported AT force is underestimated by, on average, 0.3 kilonewtons.

AT skin temperature analysis was performed on series of thermography images acquired during the testing (Fig. 3). Average temperature over the area covering 60–80% of the free tendon length was calculated throughout the recording period in 10 second intervals. The period during which the contractions were performed, was excluded from the analysis, because the AT did not remain stationary. The increase of AT skin temperature was determined individually as the difference between the 1st image frame (prior to contractions) and the maximum temperature, which was usually attained 3-7 minutes after the 1st contraction. The

increase of AT skin temperature was divided by the number of contraction cycles to yield average increase of temperature per cycle.

AT temperature gradients were analyzed from thermal images by dividing AT into three regions of equal size. This was done to confirm the findings of the pilot study that AT temperature is not influenced by heat dissipated from proximal muscles. Average temperature of each region is denoted by T_1, T_2 and T_3 and average location by x_1, x_2 and x_3, where numbers 1, 2 and 3 indicate proximal, middle and distal regions, respectively. Temperature gradients were calculated as

$$g_i = \frac{T_{i+1} - T_i}{x_{i+1} - x_i}$$

where i gets values 1 and 2.

To calculate absorbed heat, it was assumed that heat is evenly distributed over the AT and thus AT skin surface temperature equals AT core temperature. Absorbed heat ΔQ and increase of temperature ΔT are related

$$\Delta Q = mc\Delta T \quad (1)$$

Where m is a body's mass and c is specific heat capacity. AT mass was calculated assuming that geometrical shape of the AT is a thin cylindrical rod with a cross-sectional area A and length l. In that case, equation (1) transforms to

$$\Delta Q = \rho A l c \Delta T \quad (2)$$

where ρ is density of the tendon. Cross-sectional area A and length l were taken individually and tendon density ρ as well as specific heat capacity c were taken from the literature (IT'IS Foundation, 2013).

Example of the calculation of absorbed heat ΔQ for subject number 1 [Eq. (2)] is given below

$$\Delta Q = 1525 \text{ kg } m^{-3} \times 62.0 mm^2 \times 175\ mm \times 2372 \text{ J } kg^{-1}\ {}^{\circ}C^{-1} \times 0.045 {}^{\circ}C = 0.9 J$$

Kolmogorov-Smirnoff test was used to test for data normal distribution and non-parametric tests were used accordingly. Wilcoxon signed-rank test was used to test differences and Spearman's rank correlation was calculated between selected parameters. The level of significance was always set to $P<0.05$. Presented values are mean ± standard deviation (s.d.).

RESULTS

Individual AT force-elongation curves, from which elastic energy and hysteresis were calculated, are displayed in Fig. 5. The non-zero intercept of the Y-axis is due to cutting of the data at the 5% MVC force level. AT stored on average 13 ± 4 joules of energy when it was stretched and dissipated 1.5 ± 1.2 joules to heat when it was released. Thus AT hysteresis was 11 ± 10 % (Table 2).

Increase of AT skin temperature, assessed with IRT imaging, was on average 0.025 ± 0.014°C per cycle (N = 19; P < 0.001; significantly different from zero) or 0.375°C ± 0.212°C after 15 cycles (N = 19; P < 0.001). As expected, increase of AT skin temperature correlated positively with hysteresis (N = 19, R = 0.471, P = 0.042) (Fig. 6).

Dissipated heat (1.5 ± 1.2 J) was compared to absorbed heat (1.2 ± 0.7 joules), which was calculated from the relation between change in heat and change in temperature [Eq. (2)]. Dissipated and absorbed heat were not significantly different (N = 19, P = 0.629) nor did they correlate with each other (N = 19; R = 0.239; P = 0.324).

Achilles tendon temperature gradients at the 1st IRT image prior to contractions were negative and there was a significant difference between the proximal (g_1 = -0.056 ± 0.007°C m^{-1}) and distal (g_2 = -0.029 ± 0.010°C m^{-1}) mean gradients (N = 19, P = 0.001) indicating that distal parts of the free AT were cooler than proximal parts, and the difference was greater at the proximal end. However, neither proximal nor distal temperature gradient changed significantly from the 1st IRT image at any point during the monitoring period.

DISCUSSION

To our knowledge, this is the first study to simultaneously measure AT hysteresis and corresponding increase of AT skin temperature. Average increase of AT skin temperature, assessed with IRT imaging, was 0.025°C per stretch-shorten cycle or 0.375°C after 15 stretch-

shorten cycles. At the same time, force-length derived AT hysteresis was on average 11 %. As hypothesized, variation in hysteresis was correlated with variation in increase of temperature: greater hysteresis was related to greater increase of AT skin temperature. To confirm that increase of AT skin temperature was not simply due to heat generated by the muscles, skin temperature gradients along the length of the free AT were analysed. Both proximal and distal gradient remained unchanged throughout the experiment indicating no changes in heat transfer rate from along the length of the AT. Lack of heat transfer from proximal muscles to distal tendon was also supported in the pilot study, when even a 6°C increase in gastrocnemius skin temperature was not enough to increase AT skin temperature (Fig. 1). IRT imaging cannot provide numerical values of tendon hysteresis, but compared to equipment needed to deduce force-length data, IRT cameras are superior in mobility and ease of use. Thus IRT imaging may be used to follow tendon thermoregulation and possible changes in hysteresis that occur due to training (Foure et al., 2010) or tendon fatigue (Fung et al., 2009).

In the current study AT stored an average of 13 joules of strain energy during one stretch. In one legged-hopping AT has been shown to store 51 joules (Lichtwark and Wilson, 2005). One-legged hopping is an especially stressful task for the AT, as it induces twice as much strain as running (Farris et al., 2011). Since stored strain energy is proportional to the second power of elongation, doubling the strain quadruples the stored energy. This explains why AT could have stored four times the energy in one legged-hopping compared to the current study. As the

currently measured plantar flexion force – twice the body weight at 80 % of MVC – equates to vertical ground reaction force during slow running (Nilsson and Thorstensson, 1989), the current AT energies are likely to be similar to those during sustained activities such as endurance running.

We estimated the power at which tendon converts elastic strain energy to heat during running and compared that to muscle. As dissipated heat was on average 1.5 joules and AT mass was 20 grams (Table 2), we estimated that at 1.5 Hz stride frequency AT dissipates elastic energy to heat at a power of 113 W kg^{-1}. To calculate the power at which muscle converts chemical energy to heat, 4 kJ kg^{-1} km^{-1} was used as cost of transport (Cavagna and Kaneko, 1977). If a runner advances at an endurance running pace, 10 km h^{-1}, chemical energy conversion rate is 11 W kg^{-1}. This is expressed for full body mass. Assuming that chemical energy is harvested by a fraction of full body mass, i.e. 20 %, energy conversion rate becomes 56 W kg^{-1}. When running on a flat surface, it is safe to assume that all chemical energy is converted to heat, because negligible work is done against air resistance and no work is done to increase gravitational potential energy of the body. This calculation is only an approximation, but it shows that AT may dissipate elastic strain energy to heat at a rate that is comparable to chemical energy conversion rate of the muscle. Thus it is justified to argue that low hysteresis is desirable not only to save energy but also to protect tendon against heat induced damage (Alexander, 1991).

Absorbed heat raises the temperature of the tendon, to as much as 45 °C in the case of superficial digital flexors of galloping horses (Wilson and Goodship, 1994) and 41 °C in the case of AT of running humans (Farris et al., 2011). Excessive increase in temperature, known as tendon hyperthermia, has been postulated to damage the tendon (Birch et al., 1997). The current results show that there is a possibility that some tendons are at increased risk of tendon hyperthermia due to high hysteresis, although tendons in the current study did not reach hyperthermic temperatures. Thus it is not known what happens to tendon temperature over continuous cyclic stretching and shortening. It is possible that the currently observed increase of temperature continues to reach hyperthermic values, but it is equally possible that the increase of temperature levels off due to thermoregulation, and hyperthermic temperatures are never reached.

The current mean AT hysteresis was 11 %. This is slightly higher than the typical 7 % hysteresis of excised tendons (Bennett et al., 1986;Ker, 1981), but at the lower end of the published range from 3 to 35 % for human AT in vivo (Farris et al., 2011;Foure et al., 2010;Kubo et al., 2002;Lichtwark and Wilson, 2005;Maganaris and Paul, 2002;Peltonen et al., 2013;Stenroth et al., 2012). There is some evidence in both humans and animals to support the idea that tendon hysteresis may be adaptable, which explains the observed variation. One animal study found that high-stressed flexor tendons have lower hysteresis than low-stressed extensor tendons (Shadwick, 1990). Similar findings were made after an intervention, in which subjects

who underwent a training programme that consisted mainly of jumps decreased their AT hysteresis (Foure et al., 2010). Also stretching has been found to decrease AT hysteresis (Kubo et al., 2002). Thus it is possible that to maintain low hysteresis AT should be stressed more frequently than happens in today's sedentary life style. Failure to regularly stress AT may explain why high values (> 10 %) are sometimes reported for AT in vivo.

Limitations of the current study

Main limitations of the current study include measuring increase of AT temperature from the skin rather than from tendon core and determining AT hysteresis from a single head of triceps surae muscle. First, it was assumed that increase of AT skin temperature reflects whole tendon temperature change. The idea was tested by comparing calculated dissipated and absorbed heats. Absorbed heat, which was calculated based on increase of temperature (Eq [2]), was not significantly different from dissipated heat supporting the idea that increase of AT skin temperature is equal to increase of tendon temperature. However, dissipated and absorbed heats were not related as evidenced by their lack of correlation. This may have been due to large uncertainties of their variables. Second, AT comprises of the three heads of triceps surae muscle, which is assisted by deep ankle plantar flexors. Thus varying synergist activation (Finni et al., 2000;Finni et al., 2006) may induce regional strain variations that are seen as variation in hysteresis. Within-individual variation in synergist activation was treated by averaging AT force-length curves over several trials in the current study. It is also possible that synergist

activation varies between individuals, which may then lead to individual under- or overestimation of AT hysteresis. However, the correlation between AT hysteresis and increase of temperature indicates that variation in hysteresis is derived from the properties of the tendon, rather than from the behaviour of the muscles.

CONCLUSIONS

A positive correlation between AT hysteresis and increase of AT skin temperature indicates that variation in AT hysteresis may be a consequence of tendon mechanical properties rather than a measurement artefact. The finding that IRT imaging was able to detect subtle changes in AT skin temperature after 15 stretch-shorten cycles indicates that thermal cameras are a potentially useful tool to investigate AT thermoregulation and/or adaptation in hysteresis due to physical activity.

FIGURE AND LEGENDS

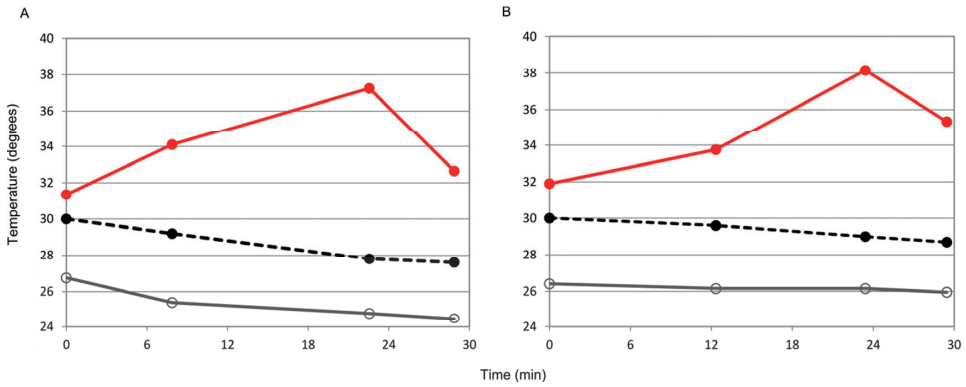

Figure 1. Pilot study on thermal convection. Skin temperatures over gastrocnemius muscle (solid line, filled circles), Soleus AT (broken line) and free AT (solid line, open circles) for two subjects (A and B). Temperatures were analysed from IRT images 1) before; 2) after gastrocnemius exposure to 41°C water; 3) after gastrocnemius exposure to 46°C water; and 4) after 5 minutes of recovery. AT = Achilles tendon, IRT = infrared thermography.

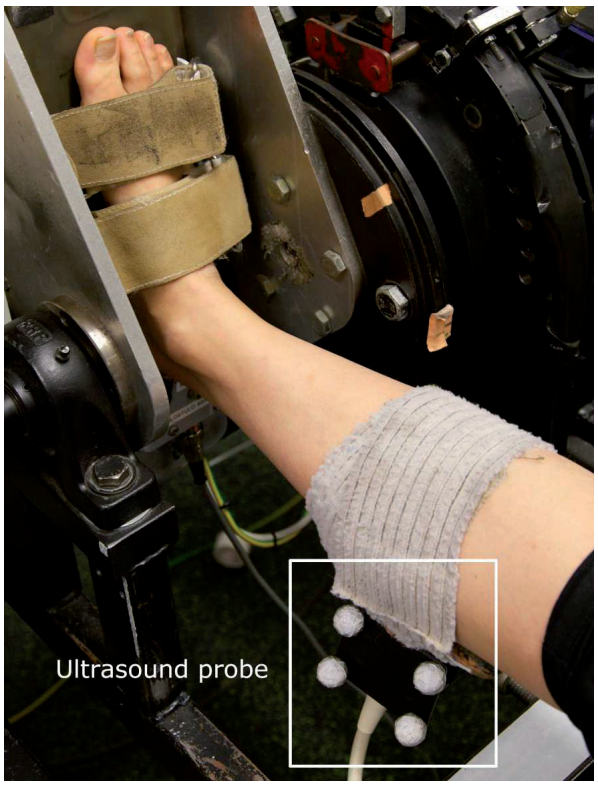

Figure 2. Illustration of ankle dynamometer setup. Subject's right leg was attached to the force pedal and secured with foot straps. Ultrasound probe was placed over medial gastrocnemius muscle and four spherical markers allowed motion tracking of the ultrasound probe. Thermal camera (not visible in the image) was placed below the leg.

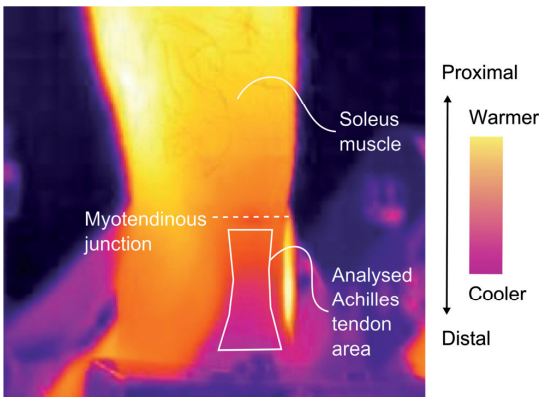

Figure 3. Example of Achilles tendon (AT) thermography image analysis. AT skin temperature was analyzed from the posterior surface of the right leg. Temperature was taken as an average of the area that covered 60–80% of free AT from calcaneus to myotendinous junction of the Soleus muscle.

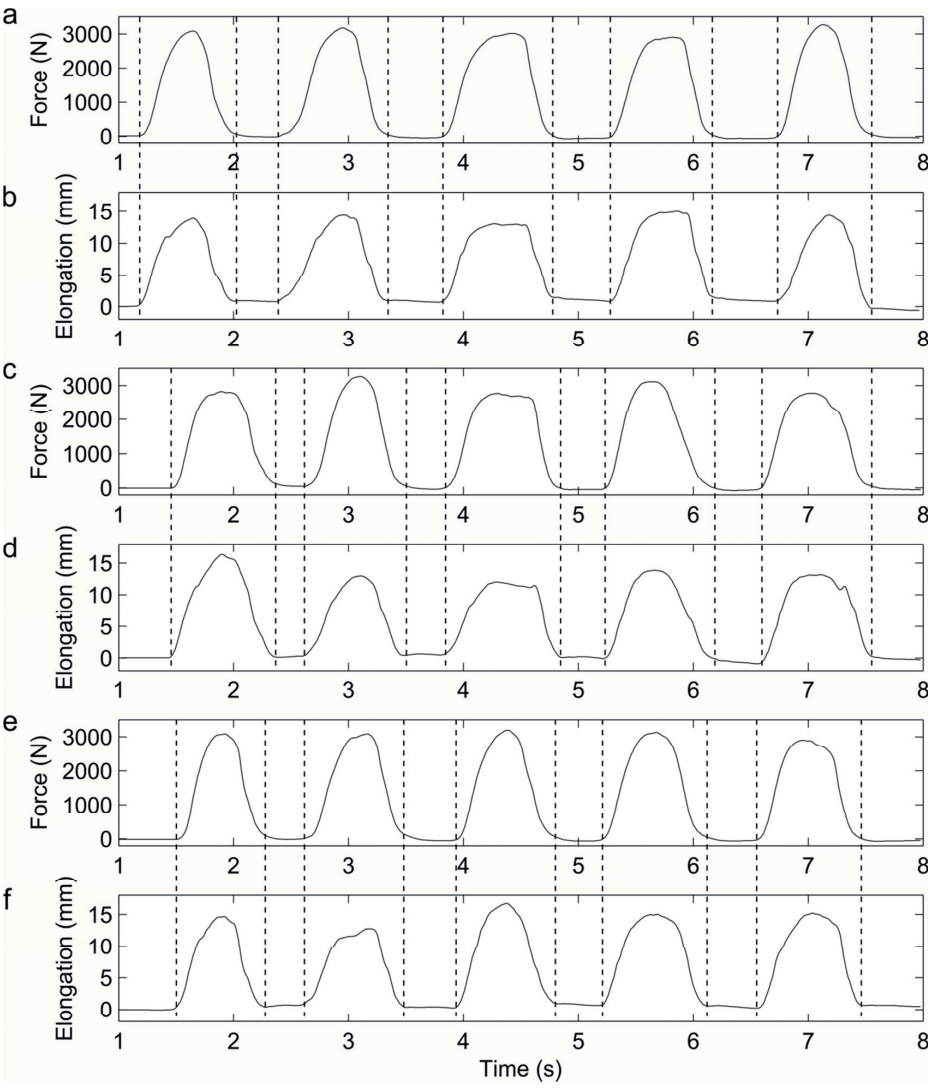

Figure 4. Example of tendon force and elongation data as a function of time from one subject. Total of 15 contractions were divided into three sets of five repetitions: (A, B) Reps 1–5. (C, D) Reps 6–10. (E, F) Reps 11–15.

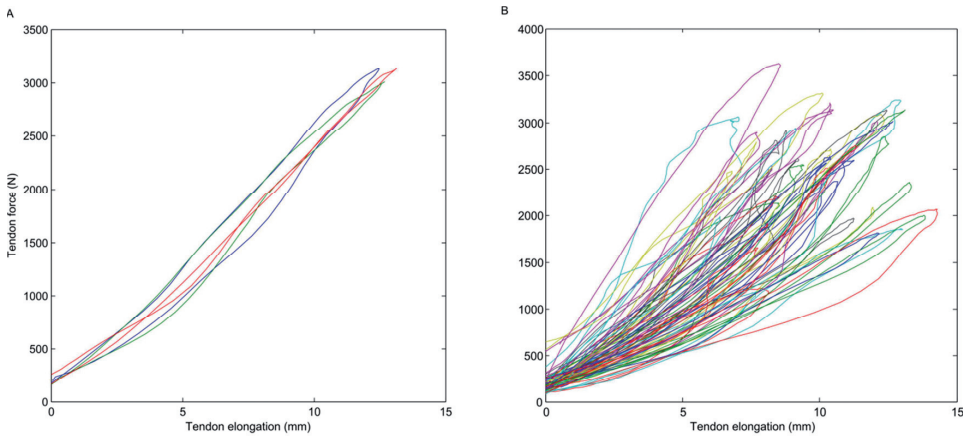

Figure 5. Achilles tendon force-elongation curves. (A) Consecutive curves from the same subjects as in Fig 4. One curve is an average of five repetitions. (B) All force-elongation curves from 19 subjects.

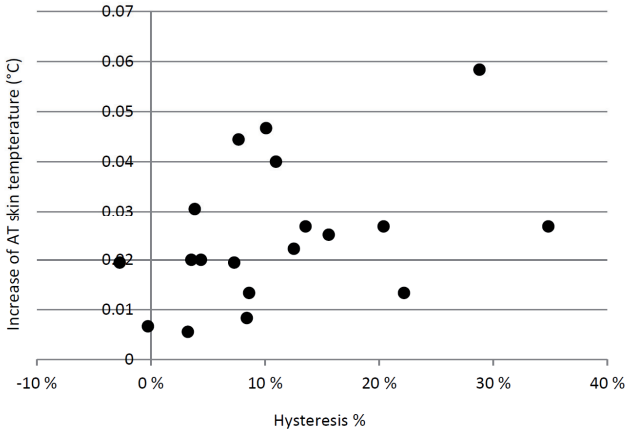

Figure 6. Achilles tendon (AT) hysteresis vs. increase of AT skin temperature.

REFERENCES

References

Alexander, R. M. (1991). Energy-saving mechanisms in walking and running. *Journal of Experimental Biology* **160,** 55-69.

Arora, N., Martins, D., Ruggerio, D., Tousimis, E., Swistel, A. J., Osborne, M. P. and Simmons, R. M. (2008). Effectiveness of a noninvasive digital infrared thermal imaging system in the detection of breast cancer. *Am. J. Surg.* **196,** 523-526.

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

Birch, H. L., Wilson, A. M. and Goodship, A. E. (1997). The effect of exercise-induced localised hyperthermia on tendon cell survival. *J. Exp. Biol.* **200,** 1703-1708.

Bouguet, J. Y. (2001). Pyramidal implementation of the Lucas Kanade feature tracker. Description of the algorithm. **2013,** 9.

Cavagna, G. A. and Kaneko, M. (1977). Mechanical work and efficiency in level walking and running. *J. Physiol.* **268,** 467--81.

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

Finni, T., Hodgson, J. A., Lai, A. M., Edgerton, V. R. and Sinha, S. (2006). Muscle synergism during isometric plantarflexion in achilles tendon rupture patients and in normal subjects revealed by velocity-encoded cine phase-contrast MRI. *Clin. Biomech.* **21,** 67-74.

Finni, T., Komi, P. V. and Lepola, V. (2000). In vivo human triceps surae and quadriceps femoris muscle function in a squat jump and counter movement jump. *Eur. J. Appl. Physiol.* **83,** 416-426.

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

Fung, D. T., Wang, V. M., Laudier, D. M., Shine, J. H., Basta-Pljakic, J., Jepsen, K. J., Schaffler, M. B. and Flatow, E. L. (2009). Subrupture tendon fatigue damage. *Journal of Orthopaedic Research* **27,** 264-273.

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

IT'IS Foundation. (2013). Tissue properties database. *The Foundation for Research on Information Technologies in Society* **2013,**.

Ker, R. F. (1981). Dynamic tensile properties of the plantaris tendon of sheep (Ovis aries). *Journal of Experimental Biology* **93,** 283-302.

Kubo, K., Kanehisa, H. and Fukunaga, T. (2002). Effect of stretching training on the viscoelastic properties of human tendon structures in vivo. *J. Appl. Physiol. (1985)* **92,** 595-601.

Kubo, K., Kawakami, Y., Kanehisa, H. and Fukunaga, T. (2002). Measurement of viscoelastic properties of tendon structures in vivo. *Scand. J. Med. Sci. Sports* **12,** 3-8.

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

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

Maganaris, C. N. and Paul, J. P. (2002). Tensile properties of the in vivo human gastrocnemius tendon. *Journal of biomech.* **35,** 1639-1646.

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

Nilsson, J. and Thorstensson, A. (1989). Ground reaction forces at different speeds of human walking and running. *Acta Physiol. Scand.* **136,** 217-227.

Peltonen, J., Cronin, N. J., Stenroth, L., Finni, T. and Avela, J. (2013). Viscoelastic properties of the Achilles tendon in vivo. *SpringerPlus* **2,** 1-8.

Peltonen, J., Cronin, N. J., Stenroth, L., Finni, T. and Avela, J. (2012). Achilles tendon stiffness is unchanged one hour after a marathon. *J. Exp. Biol.* **215,** 3665-3671.

Shadwick, R. E. (1990). Elastic energy storage in tendons: mechanical differences related to function and age. *J. Appl. Physiol.* **68,** 1033-1040.

Stenroth, L., Peltonen, J., Cronin, N. J., Sipilä, S. and Finni, T. (2012). Age-related differences in Achilles tendon properties and triceps surae muscle architecture in vivo. *Journal of Applied Physiology*.

Vainionpää, M., Tienhaara, E., Raekallio, M., Junnila, J., Snellman, M. and Vainio, O. (2012). Thermographic Imaging of the Superficial Temperature in Racing Greyhounds before and after the Race. *Scientific World Journal* **2012,**.

Wilson, A. M. and Goodship, A. E. (1994). Exercise-induced hyperthermia as a possible mechanism for tendon degeneration. *J. Biomech.* **27,** 899-905.