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Differences in properties of the triceps surae muscle-tendon unit in Maasai jumpers and Caucasian controls during repetitive jumping

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Summary

Purpose. The present study examined the anthropological features in ten Maasai males and investigated their muscle-tendon unit (MTU) function during repetitive jumping via fascicle behavior. The obtained data was compared to a group of six Caucasian males.

Methods. Ultrasonography, kinematics, kinetics and electromyography (EMG) data was recorded during two conditions; submaximal and maximal repetitive jumping.

Results. The Maasai jumpers had a longer Achilles tendon and foot than the Caucasian controls. During both conditions of repetitive jumping the Maasai jumpers had a lower jumping frequency, longer contact time, and a larger range of motion (ROM) of both the ankle, knee and hip than Caucasian controls. From submaximal to maximal condition frequency decreased, flight time was longer, and angular ROM increased for both groups. Fascicle behavior during ground contact was generally isometric for both groups, and the fascicle length of Maasai jumpers was shorter than that of Caucasian controls. The muscle-tendon unit (MTU) of both groups demonstrated a classical stretch-shortening cycle behavior during ground contact. The gastrocnemius medialis (GM) MTU length change and GM tendinous tissues length change was larger for the Maasai jumpers during both conditions compared to Caucasian controls. GM MTU length change was larger in maximal compared to submaximal condition for both groups. The EMG activation pattern of GM, SOL and VL was different between Maasai jumpers and Caucasian controls.

Conclusion. This study on Maasai jumpers revealed that they have optimized a jumping technique with a longer contact time and a larger ROM of the ankle, knee and hip than Caucasian controls. As a result of larger angular ROM the Maasai jumpers had a larger stretch of GM MTU and GM tendinous tissues. The longer AT and foot of the Maasai jumpers correlated weakly to jumping performance.

Preface

First of all, I want to express my gratitude towards my supervisor Jens Bojsen-Møller and my co-supervisor Olivier Seynnes. Your support through this whole year has been highly appreciated. Allowing me to take part in such a large project, and by challenging me to take part in planning the whole experimental setup, you have really sparked my interest in the project. Your knowledge on the subject is outstanding, and I would like to thank both of you for sharing that knowledge through great advice.

This project would never have been possible without Dirk Lund Christensen, and his knowledge on how to carry out a field study half way around the globe. Involving Jens in his larger study of the Maasai people of Tanzania, is what opened this opportunity for me in the first place, and for that I'm grateful. Before, and during the field trip to Tanzania I have learned more than I could have ever imagined. There has been a lot of work, and many late nights, as bringing a field lab to a place you have no knowledge of requires a lot of practice and many plans for worst case scenarios.

I also want to thank Vidar Jakobsen for his help in solving methodological problems regarding analysis of results from Qualisys and for his MATLAB expertise. Per Aagaard and Peter Magnussons opinion on data collection and analysis has been highly appreciated.

For a successful field trip to Tanzania I would like to thank Dirk Lund Christensen, Jens Bojsen-Møller, Olivier Seynnes, Jørgen Jensen and Andreas Skiri Refsdal. The many great Tanzanian helpers are not forgotten, and their help was absolutely needed. It was great learning to know all of you.

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Herman Hernæs, May, 2016

Contents

1. Abbreviations

AT	Achilles tendon
CSA	Cross sectional area
EMG	Surface electromyography
GM	Gastrocnemius medialis
MTU	Muscle-tendon unit
ROM	Range of motion
SOL	Soleus
SD	Standard deviation
SSC	Stretch-shortening cycle
TA	Tibialis anterior
US	Ultrasound
VL	Vastus lateralis

2. Introduction

According to their own history Maasai settled in the northern areas of Tanzania and the south of Kenya, only a few hundred years ago. They descend from Nilotic tribes that migrated south from Sudan, and assimilated with the Cushitic tribes in the area. Traditions was adopted from both tribes, but the Maasai are specifically known for their religious rituals involving chanting, dancing and repetitive jumps. Young men between approximately 15-35 years are the warriors of the tribe. It is these young men that participate in the repetitive jumps during the rituals.

Preliminary observations by Dirk Lund Christensen (Section of Global Health, University of Copenhagen, Copenhagen, Denmark) reports that during these repetitive jumps participants move their body center of mass more than 50 cm. Biomechanical analysis of the Maasai jumpers through muscle-tendon unit (MTU) behavior has never been studied. There is limited data on anthropometrical features, though at least one group has explained the features of the Maasai foot (Choi, Suh, & Seo, 2014).

There are many factors that could explain the seemingly great jumping performance of the Maasai. The most plausible explanation is that Maasai males are trained to perform in the rituals from a young age. During the field trip to Tanzania it was clear that this is also the case, and many reported that they started jumping when they were about five years old. This is only an approximation as the Maasai are unaware of their actual age.

Jumping, and repetitive jumping, are natural and complex human movements that involves most of the lower extremity muscles. During such movements the leg represents spring-like characteristics (Hobara, Kanosue, & Suzuki, 2007; Kuitunen, Ogiso, & Komi, 2011) and the stretch-shortening cycle (SSC) of muscle-tendon function can be utilized effectively (Finni, Komi, & Lepola, 2001).

The most studied jumps are squat jumps, countermovement jumps, and drop jumps, while repetitive jumps have been less studies and therefore have less existing literature (Lamontagne & Kennedy, 2013). The short contact time of the repetitive jumps allows for less range of motion (ROM) about the ankle-, knee- and hip joint than during squat jumps or countermovement jumps, with the majority of the forces generated about the knee and ankle (Finni, Komi, et al., 2001; Honeine, Schieppati, Gagey, & Do, 2013). Because of the low ROM about the knee joint, repetitive jumps rely heavily on the plantar flexor muscles of the lower leg. The plantar flexors are commonly referred to as the triceps surae complex. The two-headed biarticular gastrocnemius and the monoarticular soleus (SOL) forms this MTU together with the Achilles tendon (AT).

The jumping performance of the Maasai could also be explained by anthropometrical features. In geographical distance we don’t need to look far to find other great athletes, as the runners of Kenya and Maasai both are part of an east African population. The Kenyan endurance runners display some anthropological features of muscle and tendon in the lower leg that enables them to utilize energy efficiently during SSC movements (Sano et al., 2013; Sano et al., 2015).

When compared to physically active Caucasian males that was matched for height but not body mass, Sano et al. (2013) found that Kenyan runners had a longer AT length at rest (26,4 cm vs. 19,7 cm) and a lower gastrocnemius medialis (GM) pennation angle (20 degrees vs. 21,8 degrees). Both groups performed maximal repetitive jumps. During these jumps the Kenyan runners had a shorter ground contact time, a longer flight time, and as a consequence of the two, a greater jump height than the Caucasians (Sano et al., 2013). The seemingly efficient utilization of elastic energy during repetitive jumps was explained by a high recoil from tendinous tissues despite a smaller range of length change in both fascicles and tendinous tissues.

Sano et al. (2015) measured anthropometrics of Kenyan runners and compared the values to Japanese runners. Both groups were matched for height and weight. The Kenyan runners had a longer lower leg (39,5 cm vs. 36,7 cm), longer AT moment arm (4,47 cm vs. 3,7 cm), longer GM MTU (43,4 cm vs. 35,3 cm) and longer GM tendinous tissues (39,4 cm vs. 35,3 cm) (Sano et al., 2015). The Kenyan runners also had shorter GM fascicles (4,8 cm vs. 5,7 cm) and greater GM pennation angle (20,6 degrees vs. 16,2 degrees). During running the Kenyan runners seemed able to utilize elastic energy more efficiently as the longer AT moment arm resulted in lower AT strain and GM muscle activation allowing fascicles to work more isometrically during ground contact (Sano et al., 2015).

2.1 Purpose and hypothesis

The present investigation has two main aims:

1. To examine anthropometrical features in a group of Maasai jumpers and to investigate MTU function during repetitive jumping.
2. To compare these data to those obtained in experienced jumpers as well as in sedentary age matched adults.

From data obtained on Kenyan runners by Sano et al. (2013) and Sano et al. (2015) it is hypothesized that the Maasai jumpers has a long AT, a long AT moment arm, and that they display small fascicle length changes during the contact time of repetitive jumps.

This master thesis is part of a larger biomechanical study of the Maasai people. It is written as a scientific article with an extended theory- and methods chapter. The article will present the results of the study, and discuss these findings. In addition to this it will contain an abstract, a short introduction, and a brief description of methods. The extended theory chapter will present the current literature of the field in an organized way. First muscle, from what it is to how it works, will be explained. It is followed by a chapter presenting tendons in the same manner. From the explained parameters in these chapters the interplay of muscle and tendon during SSC movements will be presented. Lastly, I will present how this interplay influence repetitive jumping, and how ethnic differences in muscle and tendon structure affects the behavior of the MTU.

3. Theory

3.1 Muscle

Skeletal muscles have numerous functions in the body, they exert force and produce mechanical work, produce heat and assists in thermoregulation (Zatsiorsky & Prilutsky, 2012). This chapter will focus on the morphological and mechanical properties of skeletal muscle that are important for repetitive jumping. Important morphological properties of repetitive jumping are fascicle length, muscle fiber type and cross sectional area (CSA), while important mechanical factors are the force-velocity and force-length relationship of muscle contraction (Lamontagne & Kennedy, 2013; Roberts, 2002; Stafilidis & Arampatzis, 2007).

3.1.1 Morphological properties

What gives the muscle its ability to shorten, and the ability to produce mechanical work, is the contractile proteins myosin and actin. Together with the z-membrane, myosin and actin makes up the most important structures of a sarcomere. Myosin pulls on actin filaments to make muscle fibers shorten. In GM about 15000 of these sarcomeres make up a myofibril (Zatsiorsky & Prilutsky, 2012). Thousands of these myofibrils forms a muscle fiber (Lieber, 2010). The muscle fiber is enclosed by sarcolemma, and surrounded by endomysium. A number of muscle fibers form fascicles that are enclosed by perimysium. Finally, epimysium covers the entire muscle. The number of muscle fibers in GM is just above one million (Zatsiorsky & Prilutsky, 2012).

Since the early 17th century work of Nicolaus Steno it has been known that the arrangement of fascicles differs in different muscles depending on its function (Kardel & Maquet, 2013). The orientation of fascicles away from the direction of muscle force action is called the pennation angle of the fibers. The arrangement of fibers in angle to muscle force action has some advantages and some disadvantages. First of all, more fibers can be filled into a given volume of a muscle, and the total force of the muscle fibers can be greater than that in fusiform muscles of the same volume (Zatsiorsky & Prilutsky, 2012). Packing more fibers in a given volume it also a disadvantage of pennate muscles because when fibers are not aligned with the line of action only some of the fiber force contributes to muscle force. It also results in fewer sarcomeres in series which means smaller maximum displacements and velocities.

Pennation angle is subject to change during muscle contraction or joint displacement (Maganaris, Baltzopoulos, & Sargeant, 1998b; Narici et al., 1996), but also permanently as a result of ageing (as a result of growth) (Binzoni et al., 2001) or hypertrophy (Seynnes, de Boer, & Narici, 2007). During contraction pennation angle increases and fascicle length decreases as the fibers rotate.

In part due to pennation the CSA of a muscle can be expressed as both the anatomical CSA and physiological CSA. The physiological CSA is defined as “the magnitude of muscle fiber area perpendicular to the longitudinal axis of individual muscle fibers multiplied by the cosine of the angle of pennation” (Aagaard et al., 2001; Wickiewicz, Roy, Powell, & Edgerton, 1983). This means that the physiological- and anatomical CSA of a parallel fibered muscle always will be the same, and that for pennate muscles the physiological CSA will always be larger than the anatomical CSA, as at a given muscle volume fiber pennation increases the physiological CSA.

Muscle force arises from the forces exerted by fibers, and since the physiological CSA represents the maximum number of actin and myosin cross-bridges in parallel, the maximal force that muscle fibers together can exert is proportional to the muscles physiological CSA (Aagaard et al., 2001; Zatsiorsky & Prilutsky, 2012). Determination of physiological CSA is not simple, and it is usually determined from muscle volume and fiber length. The anatomical CSA on the other hand is much easier to determine, as it is perpendicular to the tendons. Muscle thickness in pennate muscle is defined as the shortest distance between the tendons of origin and insertion measured in the plane of anatomical cross-section (Zatsiorsky & Prilutsky, 2012).

3.1.2 Mechanical properties

The length and contraction velocity of a muscle is directly related to the maximum force that can be generated as the force-length curve of a muscle is affected by the overlap of myosin and actin in sarcomeres, which determines the active cross-bridges. Sarcomeres are the smallest contractile elements of skeletal muscles, and they connect in series along the muscle fiber (Zatsiorsky & Prilutsky, 2012). Fibers can span the entire length of a fascicle, or end somewhere in the middle (Hijikata & Ishikawa, 1997). At the optimal overlap of myosin and actin maximum force can be created. As the muscle contracts, myosin overlaps actin and the result is fewer active cross-bridges that results in a rapid reduction in force. As a muscle is stretched from the optimal overlap fewer myosin heads can pull on actin filaments and force is reduced (Figure 2). It is important to note that this is a simplification of the properties of the fibers in muscle, and that elastic properties and changes of moment arm about joints will affect the curve. Abe, Kumagai, and Brechue (2000) found differences in fiber length, pennation angle and CSA between sprinters, distance runners and controls, with sprinters having longer fascicles and less pennation angle than both distance runners and controls. These characteristics are important for the maximal shortening velocity, and are therefore important for faster sprint performance.

The force-velocity relationship, or Hill's curve, of a contracting muscle was first described by Hill (1938). It describes the force production of both concentric and eccentric muscle contractions (Figure 1). As the velocity of a concentric muscle contraction increases, muscle force decreases. Eccentric contraction forces rise with increasing velocity.

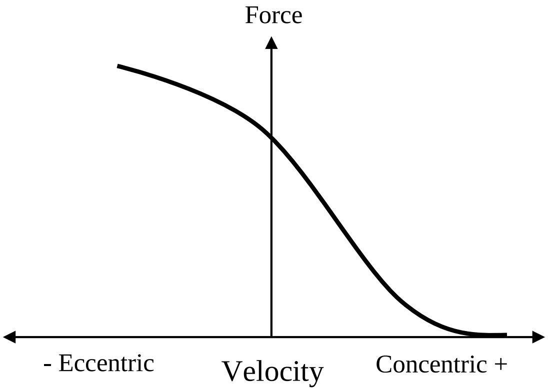

Figure 1: *The force-velocity relationship of muscle. During concentric contractions force declines as velocity increase. In eccentric contractions force increase as velocity increase.*

In addition to being the main motor of joint movement, muscle tissue also displays elastic properties. The elastic properties are called passive properties of muscle. Passive properties of muscle are thought to be affected by many structures and mechanisms. Tendons are the largest contributor to series elasticity, but some series elastic components are found within the cross-bridges of the muscle, in the crosslinks between myosin and actin, and from the stretching of non-contractile proteins of cytoskeletons (Gajdosik, 2001; Roberts, 2002). Parallel elastic components include the deformation of connective tissues located within and surrounding the muscle (Gajdosik, 2001; Horowits, Kempner, Bisher, & Podolsky, 1986). If a muscle is stretched from its resting length, passive properties of muscle contribute to the force produced (Figure 2).

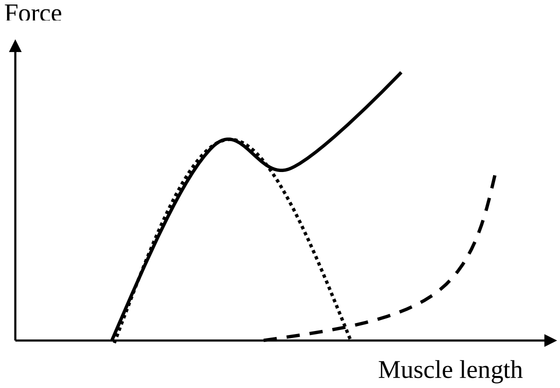

Figure 2: *The force-length relationship of muscle. The solid line represents the force-length relationship of muscle taking passive elastic properties into account. The dotted line represents the force-length relationship of a single muscle fiber, and the dashed line elastic properties. The combined force of muscle fibers and elastic properties are represented by the solid line.*

3.2 Tendon

In order to understand tendon function during repetitive jumping we need knowledge of tendon structure and its mechanical properties.

Human tendons connect muscle to bone, and allows for the transfer of muscle force into movement about joints. Tendons are known to be the major source of series elastic elements, and to influence the mechanics of muscle, but our knowledge of the exact role of the tendon during human movements has not been known until recent years (Kubo, Kawakami, & Fukunaga, 1999). The advances in ultrasound (US) technology has made it possible to study tendons *in vivo*, and in that way study the elongation of tendon in relation to muscle action. This has enhanced our knowledge from *in vitro* studies using human cadavers and animals to determine tendon mechanics (Bertolotto et al., 1995; Kubo et al., 1999).

Like muscle, tendons are organized hierarchical form small collagen fibrils to the entire tendon. Only three types of collagen are fiber-forming. Type I makes up 90% of the collagen in the body and is the primary collagen in tendons (Kirkendall & Garrett, 1997). The other non-fiber-forming collagen plays important roles in regulating fiber diameter, healing tendons and lubricating tendon fibers (Wang, Guo, & Li, 2012). In AT fibrils are between 30-130 nm in diameter, and are oriented in all three dimensions inside a fiber (Bertolotto et al., 1995; Józsa & Kannus, 1997). A bunch of these small collagen fibrils form a collagen fiber, which is the smallest collagenous structure that can be tested mechanically (Józsa & Kannus, 1997; Kannus, 2000). The fibers are bound together by the endotenon to form a subfascicle, and a group of subfascicles form a fascicle (Butler, Grood, Noyes, & Zernicke, 1978; Józsa & Kannus, 1997; Kannus, 2000). Groups of fascicles form tertiary bundles, and tertiary bundles make up tendons (Butler et al., 1978; Józsa & Kannus, 1997) that are surrounded by the epitenon (Kannus, 2000).

Collagen and elastin make up most of the dry mass of the tendon, 65-80% and 1-2%, respectively (Kannus, 2000; Kirkendall & Garrett, 1997). The fibrils forming collagen is one of three ground substances making up the extracellular matrix of tendons. Proteoglycans are found within and between collagen fibrils and fibers. They can hold water up to 50 times their weight, and enables the collagen fibrils to withstand high compressive and tensile forces (Józsa & Kannus, 1997). The functions of all matrix glycoproteins in tendons are not yet established, but the role of elastin is to allow tendons to return to their pre-stretched lengths after loading (Wang et al., 2012). In addition, glycoproteins heal tendon and enhance its mechanical stability (Józsa & Kannus, 1997; Wang et al., 2012).

The cells of the tendon are mostly tenocytes and tenoblasts. They are elongated fibrocytes and fibroblasts located between collagen fibers (Kannus, 2000). Tenocytes are metabolically active cells that serve a main purpose of repairing and maintaining the structure and composition of the tendon by producing collagen, elastin, matrix precursors and proteoglycans (Józsa & Kannus, 1997; Kirkendall & Garrett, 1997; Wang et al., 2012).

Tendons possess viscoelastic properties, meaning that their mechanical behavior depends on the rate of mechanical strain. This enables storing and efficient release of elastic energy by stretch and rapid recoil of the tendon. The result is minimal energy loss and deformation. This is dependent on the mechanical strain the tendon is subject to. Tendons are more deformable at low strain rates and less at high strain rates, affecting their energy absorption and effectivity (Wang et al., 2012). Viscous, or fluid-like, substances do not return to its original length or shape after being exposed to stress, while elastic substances return to its original form after being exposed to stress (Józsa & Kannus, 1997). Residual deformation of an elastic material is explained by the plasticity of the material.

Stress (psi; MPa) is defined as the force subjected by the tendon divided by its CSA, and strain (%) as the total lengthening of the tendon divided by its original length (Butler et al., 1978). The relationship between stress and strain of a material is called Hooke’s law (Viidik, 1973). The stress-strain curve of tendons is affected by the collagen fibrils three-dimensional orientation inside the fibers, and of the fibers itself (Butler et al., 1978). This complex structure gives tendons a wave-form, and protects the fibers from damage and disconnection of fibers from applied forces of various directions (Kannus, 2000). It also gives tendon the classical toe region of the stress-strain curve (Butler et al., 1978; Viidik, 1973) (Figure 3).

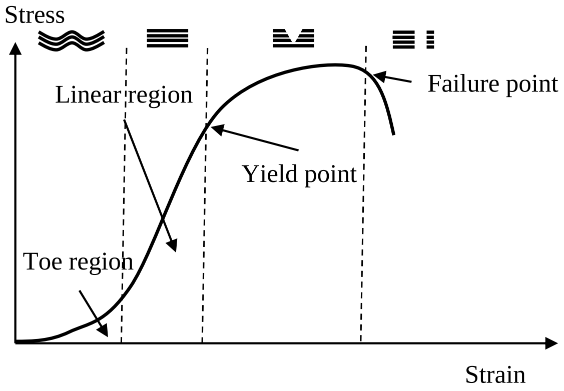

Figure 3 *The stress-strain relationship of tendon. The linear region represents the elastic region of tendon. The area from yield-point to failure point is the plastic region of tendon.*

At the toe region of the stress-strain curve fibers are stretched from the wave-form to run in parallel. Until fibers are parallel, tendon strain increases at a higher rate than the stress. The strain at the end of this region varies between 1,5-4% (Butler et al., 1978; Viidik, 1973). Following the toe region is a linear region where the tendon shows a linear response to stress. Continuous elongation of tendon in this region gives a stiffer tissue. The slope of this curve is called Young's modulus (Viidik, 1973). The strain limit of this region is reported to be between 2-5% (Butler et al., 1978). The linear region continues until a yield point where fiber failures occur in an unpredictable manner. Strain values of 3-8% are reported in this region (Butler et al., 1978). As maximum load is attained, complete failure occurs. The load-bearing ability of the tendon is then rapidly lost. It is important to note that the mentioned strain values correspond to collagen fibers tested alone, whereas the value for whole tendons reported in *in vitro* studies may be as high as 20-50% in the linear region (Butler et al., 1978).

Tendon stiffness (Nmm^{-1}) is expressed as the change in tendon force to the change in tendon length. It is the inverse parameter of tendon compliance (mmN^{-1}). Both tendon stiffness and tendon compliance is calculated in the linear region of the stress-strain curve. Aforementioned the slope of the stress-strain curve in the linear region is called Young's modulus. Young's modulus is tendon stiffness normalized to tendon CSA and length. As Young's modulus describes material properties of tendons it makes it possible to compare tendons of different dimensions.

The function of tendons is highly dependent on tendon stiffness. Tendons can, like the AT, be long and relatively thin. The tendons of the fingers are also long and thin, but the function of tendons in fingers and the AT are very different. Due to the difference in dimension of the tendons their force-elongation curves looks very different. If two tendons with different CSA and otherwise identical features was stretched to the same length, more force would be required to stretch the tendon with the largest CSA. As a result, the stiffness of the AT enables it to store and release elastic, while the stiffness of finger tendons is optimized for precision movement. The AT displays 4,2-4,9% strain, with corresponding forces and stresses of 875-2000 N and 29-32MPa, respectively (Kongsgaard, Nielsen, Hegnsvad, Aagaard, & Magnusson, 2011; Maganaris, 2002). This gives high modulus values of about 1-2Gpa (Kongsgaard et al., 2011; Maganaris, 2002).

AT spans from calcaneus to gastrocnemius. AT is separated into free AT and AT aponeurosis, as it connects to SOL distal of gastrocnemius. From gastrocnemius it twists as it descends towards calcaneus. This twisting starts proximal of the insertion of SOL and plays an important role in understanding tendon mechanics and function of AT (Józsa & Kannus, 1997). This complex tendon structure gives the tendon a different CSA along its path, from 76mm^2 proximally, 67mm^2 midway and 81mm^2 distally (Kongsgaard et al., 2011). As tendons are mechanoresponsive, which means that they have the ability to alter characteristics in response to loading conditions like immobilization, exercise, ageing and medications (Couppe et al., 2008; Kallinen & Suominen, 1994; Kirkendall & Garrett, 1997; Wang et al., 2012; Woo, Gomez, Woo, & Akeson, 1982), the size of the AT shows great variation. Normal values of CSA are in the range of 50-80 mm^2 (Kallinen & Suominen, 1994; Kongsgaard et al., 2011; Lichtwark & Wilson, 2005). Despite being a large tendon the free AT is short, only about 5,5cm (Kongsgaard et al., 2011), but wide and thick, around 12 mm and 5-6 mm for normal sedentary men, respectively (Fornage, 1986; Kallinen & Suominen, 1994)

3.3 Repetitive jumping

3.3.1 Stretch-shortening cycle

Exploring the mechanics of muscle during natural human locomotion is a continuous challenge (Ishikawa & Komi, 2008). Muscle and tendon due not behave the same during movement, but their function are tightly integrated (Roberts, 2002).

By definition SSC refers to a pre-activated muscle, which undergoes stretching during the braking phase before shortening during the push-off phase (Ishikawa & Komi, 2008). During the braking phase the leg is first compressed. This enables the storage of elastic energy in the tendon that can be used as mechanical energy during the subsequent push-off phase (Kuitunen et al., 2011; Roberts, 2002). The velocities of which MTUs shorten and lengthen would be mechanically unfavorable for muscle fibers functioning alone (Roberts, 2002). Not only does SSC reduce muscular work, but the tendon stretch and recoil allows muscle fibers to operate nearly isometrically (Roberts, 2002; Stafilidis & Arampatzis, 2007).

When accounting for the MTU as a whole, not just muscle and tendon properties separate, the classic Hill curve changes. The MTU system experience negative work during the braking phase before rapidly releasing elastic energy from tendinous tissues during the push-off phase resulting in a higher force than muscle action alone (Figure 4) (Finni, Ikegawa, Lepola, & Komi, 2001; Finni, Ikegawa, Lepola, & Komi, 2003; Ishikawa, Finni, & Komi, 2003; Komi, 2000).

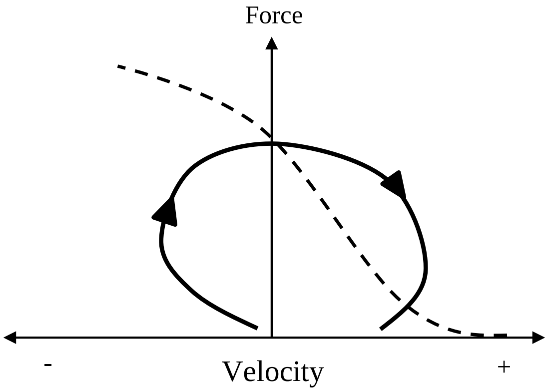

Figure 4: *The force-velocity relationship during ground contact of an SSC movement. Upward deflection signifies stretching, and downward deflection signifies shortening of the MTU.*

Repetitive jumping is a natural SSC movement that involves most lower-limb muscles, but unlike the squat jump or countermovement jump it is characterized by a short contact time, that gives small joint ROM about the hip and knee. This makes repetitive jumping ideal for investigating the plantar flexors of the shank, as they mostly contribute to the moments produced about the ankle joint (Finni, Komi, et al., 2001). Compared to other types of jumps it is also particularly effective for studying biarticular muscles, like GM, as they are easier to interpret when movement about one of the joints is limited (Lamontagne & Kennedy, 2013). Even though joint ROM is limited compared to squat jumps and countermovement jumps, interpreting MTU behavior during repetitive jumps is not easy. The function of MTU differ not only depending on joint ROM, but contact time, jump frequency and jump height as well (Finni, Komi, et al., 2001). Muscle-tendon function is also highly affected by the interaction and interplay of muscle contraction and tendon stretch (Sano et al., 2013) and the corresponding moment arm (Scholz, Bobbert, van Soest, Clark, & van Heerden, 2008).

3.3.2 Joint angles

During repetitive jumping joint ROM and joint angle during ground contact is affected by the jumping frequency (Arampatzis, Schade, Walsh, & Bruggemann, 2001; Hobara et al., 2010; Kuitunen et al., 2011). If a change in jump frequency affects the total leg stiffness of the spring system is debated. Arampatzis et al. (2001) and Hobara et al. (2010) found that with an increase in jumping frequency the total leg stiffness increased, while Kuitunen et al. (2011) reported that frequency had no effect on the leg stiffness. Kuitunen et al. (2011) tested physically active males at different jumping frequencies. They found that ankle angle at ground contact (about 130 degrees) was not affected by a change in jump frequency from 2,9 Hz to 1,8 Hz, but the ROM was increased from 18 to 29 degrees resulting in a change of minimum ankle angle from 111 to 103 degrees (Kuitunen et al., 2011). Knee angle was affected both at ground contact and during the contact phase. The knee joint at ground contact was in a more extended position at higher frequencies compared to lower (162 degrees vs. 151 degrees) with an increased ROM at higher frequencies (6 degrees vs. 11 degrees) (Kuitunen et al., 2011). For normal non-trained males during repetitive jumping at different frequencies Hobara et al. (2010) similarly found that ankle angle at ground contact (about 120 degrees) was not affected by a change in frequency from 1,5 Hz to 3,0 Hz, and that the ROM (36 degrees vs. 14 degrees) was reduced with increasing frequency (Hobara et al., 2010), resulting in a larger change of minimum ankle angle than reported for active males by Kuitunen et al. (2011) of about 106 degrees to 84 degrees. The knee angle during ground contact of about 155 degrees did not change with frequency, but an increased frequency resulted in less ROM (30 degrees vs. 4 degrees) (Hobara et al., 2010). At the highest frequency this is a higher ROM than that of the physically active males (11 degrees vs. 30 degrees). In addition to ankle- and knee joint angles Hobara et al. (2010) also reported hip angles. Hip angles were subject to change at touchdown from 165 degrees to 172 degrees as frequency changed from low to high, with the ROM reduced from 17 to 2 degrees. Overall there seems to be agreement that with increased frequency, joint ROM is reduced. During submaximal and maximal repetitive movements, the fascicle contraction is small to none during ground contact and a larger stretch of the MTU results in a larger stretch of the tendinous tissues. This enables a larger storage and release of elastic energy from tendinous tissues. In addition, the increased knee ROM as frequency is decreased is not surprising, as the intensity is higher and knee extensor muscles, like vastus lateralis (VL), has a greater muscle volume, longer fibers and shorter tendons than GM and therefore is advantageous for doing mechanical work (Kuitunen et al., 2011). This also applies for the gluteus maximus and hamstrings, the main muscles active in extending the hip. In summary, an increase in intensity results in a larger ROM of the ankle, knee and hip joint that is advantageous for storage and release of elastic energy from the AT, and for the knee and hip joint extensors to produce mechanical work.

3.3.3 Ground contact time and flight time

As frequency is increased from 1,8 Hz to 2,9 Hz, contact time is reduced from 0,22 seconds to 0,17 seconds with a reduction in flight time from 0,389 seconds to 0,122 seconds (Kuitunen et al., 2011). These data are similar to Hobara et al. (2010) reporting a reduction of contact time from 0,26 to 0,18 seconds and a reduction in flight time from 0,41-0,15 seconds (1,5 Hz vs. 3,0 Hz) and Hobara et al. (2007) reporting 0,238 seconds of contact time at a preferred frequency of 2,12 Hz and a flight time of 0,235 seconds. Other data reports only small differences in contact time from 2 Hz to maximal repetitive jump (0,171 vs. 0,181 seconds) for normal active men (Moritani, Oddsson, & Thorstensson, 1991). As jumping frequency is reduced, either the ground contact time, flight time or both has to increase. As ROM seems to increase as frequency is reduced, an increased contact time would be a reasonable explanation as it would give more time for increasing the ROM.

3.3.4 Muscle-tendon unit behavior

During repetitive jumping the GM MTU behavior represents a SSC behavior (Belli & Bosco, 1992; Lichtwark & Wilson, 2005; Sano et al., 2013; Sano et al., 2015). This classic behavior involves a stretching of the MTU during braking and a shortening during the following push-off.

Fascicle behavior during cyclic movements like walking, running or repetitive jumping has been possible to observe with the improvement of technologies like US.

Fukashiro, Komi, Jarvinen, and Miyashita (1995) reported ground reaction forces in submaximal repetitive jumping of 3026 N (kgms^{-1}) with corresponding values of 2011 N and 2146 N for squat jumps and countermovement jumps, respectively. These values are twice as high as the ones reported by Fukashiro, Komi, Jarvinen, and Miyashita

(1993), but the relationship between them are similar. The result of a higher ground reaction force is a greater stretching of the AT and a far greater load generated in AT (Fukashiro et al., 1995). This makes it clear that the elastic energy stored in AT contributes to a major part of the positive work generated during repetitive jumping.

There seems to be agreement of the major contribution the AT serves during repetitive jumping, but when looking at fascicle behavior there is less agreement. During the maximal repetitive jumps performed by one former male athlete Fukashiro et al. (1995) reported a large stretch in GM fascicle length of 32 mm during the stance phase. This represented a stretch in fascicles of 70 %, almost the same value as that of the tendon (Fukashiro et al., 1995). This is in agreement with the results of Belli and Bosco (1992) that reported a large stretch of contractile components during submaximal repetitive jumping for male athletes. Both these papers are more than 20 years old, and contractile components were only calculated and not measured *in vivo*. With the modern and more accurate methods of recent papers using US to record fascicle movement, it seems that this is an overestimation of fascicle length change during repetitive jumping. More recent investigations of submaximal and maximal SSC movement reports small to no change of fascicle length during ground contact (Sano et al., 2013; Sano et al., 2015). Even during one legged hopping, which is a more vigorous activity, only small changes in muscle length was observed (Lichtwark & Wilson, 2005). The total amount of stretch in the muscle belly was reported as 20 % of that of the AT. It is important to notice that these 20 % include the stretching of aponeurosis, tendon insertions proximal of the muscle belly, and in the fibers itself (Lichtwark & Wilson, 2005). As fascicle length is rather isometric during the stance phase of repetitive jumping most of the stretch of the MTU relates to stretch of the tendinous tissues (Lichtwark & Wilson, 2005; Sano et al., 2013; Sano et al., 2015).

3.3.5 Moment arm

In addition to the properties of contractile and elastic components of the MTU, the lever arm about the joint of which the MTU creates movement plays a significant role. Joint moment produced by a muscle is the product of the force carried by the muscles, tendon, and the moment arm of the MTU about the center of rotation (Baxter & Piazza, 2014). For the GM MTU the major lever arm is the distal AT moment arm. It can influence locomotor performance in complex ways (Baxter, Novack, Van Werkhoven, Pennell, & Piazza, 2012), but its role is not fully understood.

AT moment arm is the shortest distance from the line of action of the AT to the center of rotation of the ankle (Baxter et al., 2012). The center of rotation in the horizontal plane runs through, and close to the center, of both malleoli (Lundberg, Svensson, Nemeth, & Selvik, 1989). It also changes with the degrees of plantar flexion (Maganaris, Baltzopoulos, & Sargeant, 1998a). There are many ways to determine the length of the AT moment arm; magnetic resonance imaging (MRI) (Baxter et al., 2012; Maganaris et al., 1998a), US (Fath, Blazevich, Waugh, Miller, & Korff, 2010), x-ray (Lundberg et al., 1989), or from pictures using a measure board (Scholz et al., 2008). Using the measure board, the AT moment arm can, because the center of rotation runs close to the center of both malleolus, be defined as the mean distance from both the malleolus to the posterior aspect of AT (Scholz et al., 2008).

The length of the AT moment arm will influence the moment about the ankle joint (Baxter et al., 2012; Scholz et al., 2008). A long moment arm result in more plantar flexor moment for a given muscle force. At the same time, as we know from the force-velocity property of muscle, it shortens more rapidly during the same joint rotation and thus producing less force (Baxter et al., 2012; Lee & Piazza, 2009). In SSC movements the moment arm therefore impact the jumping height (Scholz et al., 2008). The effect of a short moment arm is claimed to have a greater impact on the amount of elastic energy stored in a tendon than the mechanical properties of the tendon itself (Scholz et al., 2008).

3.3.6 Electromyography

Surface electromyograpgy (EMG) is an established and accepted method of recording the action potential of skeletal muscle (Clarys & Cabri, 1993). When muscle cells are activated by the nervous system the electrical potential of muscle cells is changed, and it is this signal an electromyograph detects.

The magnitude of EMG is only relevant to a reference EMG from contraction of the same muscle (Burden, 2010), that is then expressed as a proportion of the reference EMG, most commonly as a percentage (Clarys & Cabri, 1993). One of the most frequently used procedures of normalizing EMG is to compare EMG activity during a given contraction to EMG activity during an isometric maximal voluntary contraction (Burden, 2010). Normalization of EMG to peak amplitude during an isometric maximal voluntary contraction is also the method recommended by the SENIAM (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles) project.

If no reliable maximal EMG values can be recorded through a maximal voluntary contraction ratios of EMG activation can be used. Two important aspects of SSC movements is pre-activation and activation of the muscles during a functional phase of a given movement (Komi, 2000). The functional phase of repetitive jumping is the contact time. During ground contact two different behaviors of the MTU occur, the stretch during braking and the shortening during take-off. As MTU behavior is so different during braking and push-off one would expect the muscle activation to also be different.

When testing Kenyan runners, Sano et al. (2013) and Sano et al. (2015) could not obtain any reliable maximal isometric contractions, and data were therefore presented as ratios of braking to pre-activation and push-off to braking. To be able to compare the activation patterns of the Maasai jumpers to the Kenyan runners, data are presented as ratios of braking to pre-activation and push-off to braking for this thesis. In light of this the theory on EMG activation will be limited to ratios of activation.

When looking at the braking phase in relation to pre-activation and push-off phase for GM and SOL, activation is higher during braking (Ishikawa, Komi, Grey, Lepola, & Bruggemann, 2005; Moritani et al., 1991; Sano et al., 2013). This is an important activity pattern of the triceps surae as pre-activation followed by a large activation during the braking phase and a reduced activation during the push-off phase can enhance the lengthening of the AT for storage and release of elastic energy (Ishikawa & Komi, 2008; Komi, 2000). During the braking phase muscle activation is lower when the muscle is operating eccentrically or isometrically, and in the following push-off phase the EMG level increases as a function of shortening velocity (Sano et al., 2015). EMG activation of the antagonist tibialis anterior (TA) during the different phases of SSC movement is rather stabile, resulting in ratios of around one (Sano et al., 2013; Sano et al., 2015).

3.3.7 Kenyan runners

As the Maasai is a sub-population living in east Africa one could think that the Maasai possess similar anthropometric features and the same muscle and tendon properties as other populations from the same area. Sano et al. (2013) and Sano et al. (2015) tested Kenyan runners against active Caucasian males and Japanese male runners, respectively. While the Japanese runners were matched for height and weight, the Caucasian subjects were only matched for height as the body mass of the Kenyan runners were low (about 58 kg). Compared to Caucasian subjects the Kenyan runners had a longer AT length at rest (26,4 cm vs. 19,7 cm) and a lower GM pennation angle (20 degrees vs. 21,8 degrees) (Sano et al., 2013). The longer AT length only correlated weakly to jumping performance. This is not surprising as AT moment arm (Scholz et al., 2008), and the interaction of muscle and tendon is known to affect jumping performance to a greater degree than AT length itself. During their more recent paper with Japanese runners they included measures of AT moment arm and analyzed its effect on energy transfer from tendinous tissues. The moment arm of the Kenyan runners was found to be longer than the Japanese runners (4,47 cm vs 3,70 cm, respectively). This in combination with a longer AT tendon was found to result in lower AT strain, less GM muscle activation and a reduction of fascicle length during running (Sano et al., 2015). Using the same calculations for MTU length and tendinous tissues length as described in “4.5 Analysis and statistics”, a longer lower leg length with a corresponding shorter GM fascicle length and greater pennation angle of the Kenyans compared to the Japanese resulted in longer GM MTU and GM tendinous tissues. (Sano et al., 2015).

During maximal repetitive jumping the Kenyan runners had a shorter contact time and a longer flight time than the Caucasian controls. As a consequence of this their jump height calculated from flight time was 73,7±59,2 % greater (Sano et al., 2013).

The activation pattern of SOL and GM EMG was also found to be different. The push-off to braking SOL EMG ratio was lower in Kenyans than the controls. The Kenyans also had a strong GM EMG signal during braking, and their braking to pre-activation ratio was significantly larger than that of the Caucasians. This activation pattern is as mentioned related to the lengthening of AT and its ability to store and release elastic energy, and is in that way associated with higher rebound performance (Sano et al., 2013).

During both maximal repetitive jumping (Sano et al., 2013) and during running (Sano et al., 2015) it was concluded that the Kenyans expressed unique muscle-tendinous characteristics enabling them to utilize elastic energy effectively during SSC movements. This was explained by a efficiency in the use of the recoil of elastic energy from tendinous tissues during repetitive jumping despite an overall smaller range of length change of both fascicles and tendinous tissues during ground contact during repetitive jumping (Sano et al., 2013), and by a longer AT moment arm that resulted in lower AT strain and GM muscle activation during running allowing fascicles to work more isometrically during the ground contact phase of running (Sano et al., 2015).

4. Methods

The following study is part of a larger study on the Maasai people. The description of methods focuses on procedures relevant for the purpose of the present thesis. For description of the full experimental trial see Appendix 2.

4.1 Design

This study was an experimental non-invasive study investigating the MTU of GM during repetitive jumping.

4.2 Subjects

The Norwegian subjects was recruited by oral and written communications. Maasai subjects were recruited through local project collaborators (via principal investigator Dirk Lund Christensen, Section of Global Health, University of Copenhagen) in the Monduli region of Tanzania.

Subjects were divided in three groups: 1) Maasai (n=20), 2) Norwegian jumping athletes (n=4), 3) control group (n=4). Because of the time constrains related to the departure to Tanzania ten random Maasai jumpers, three random Norwegian athletes and three random controls are analyzed. Due to the small number of subjects in both the Norwegian jumping athlete group and the control group, the two groups were merged. Statistical tests between the jumpers and controls revealed no significant differences between them. Maasai subjects were young athletic males from traditional Maasai communities. All Caucasian subjects were students from the Norwegian School of Sport Sciences. The experienced jumpers were still active elite athletes on national level and had been active in different jumping activities, depending on their sport, their whole life. The sports were sprinting, volleyball, basketball and long jump. The subjects in the control group were all active in sports, primarily strength training, but not in jumping movements. As the Caucasian subjects were all active in sports they were told to restrain from heavy strength training the day before testing.

All subjects signed a written informed consent form (Appendix 3). One form was made in Norwegian for the Norwegian participants, and one in Swahili for the Maasai participants. Both forms contained information about the purpose of the study, risks associated with participating and information about anonymization of data. As the study was non-invasive and the participants was not fatigued during jumps the risk associated was minimal.

The study was performed in accordance with the guidelines of the Declaration of Helsinki and was examined by relevant ethics committees in Norway and Tanzania (Appendix 4). Lastly, all subjects were informed that they could withdraw from the study at any time without having to give any reason.

4.3 Measurements

4.3.1 Anthropometric measures

Height was measured in a standing position to the nearest 0,1cm with a portable stadiometer (Meterex II, D97, UNICEF, Copenhagen, Denmark). Bodyweight was measured to the nearest 0,1kg with a portable high precision scale (type BWB-800 SMA, Tanita, Tokyo, Japan).

Tendon length was measured with the subjects lying in a supine position on a bench with the bench reaching the middle of their shank. An US system, LogicScan 128 (EXT-1Z, Telemed, Lithuania), with a linear 60 mm transducer (scanning frequency: 12MHz, HL9.0/60/128Z-2, Telemed, Lithuania) was used to find the insertion on calcaneus. The probe was moved proximally to the first insertion of SOL on AT. Both points were marked with a pen, and the distance measured externally. This is considered the free AT length. AT aponeurosis length was found moving the US probe proximally of SOL insertion to GM insertion on AT. The insertion point was marked with a pen and the distance from SOL insertion to GM insertion was measured externally (Stafilidis & Arampatzis, 2007). US pictures of resting muscle architecture of GM were acquired at the lower 30 % of muscle length in the same supine position.

Pictures used to calculate AT moment arm, foot length, and toe length was found using a custom built measurement board (Scholz et al., 2008) (Figure 5). Subjects took a standing position on top of the board with their right foot, first aligning the lateral part of the foot with the edge of the plate, and later the medial. The tip of both the lateral- and medial malleolus and the tip of the first metatarsal was marked with a pen. Pictures were taken of the foot from distance with different centers of the photo to minimize distortion of the image. Lower leg circumference was found by measuring the circumference at nine different lengths of the lower leg (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%).

Figure 5: *Zoomed-in lateral and medial view of the right foot of one Maasai subject on the custom built measurement board. From these pictures AT moment arm was calculated. Foot length was calculated form medial view pictures with the tip of the toe at center of image. Toe length was calculated from medial view pictures with the hallux at center of image. The images were taken from distance with different centers to minimize distortion.*

4.3.2 Kinematic measures

Two-dimensional kinematic data were obtained from reflective markers placed on six bony anatomical landmarks (acromion, trochanter major, lateral epicondyle of femur, lateral malleolus, calcaneus and fifth metatarsal) using three Oqus 400 cameras (Qualisys, AB, Gothenburg, Sweden) (Figure 6 and 7). The markers had a diameter of 12 mm. Three cameras were used instead of two to make sure each marker were captured at all timeframes. Sampling frequency was set to 300 Hz. Calibration of the recorded area was done using a reference frame on the force plate, and a calibration-wand. Motion data was exported directly to Qualisys Track Manager (Qualisys, AB, Gothenburg, Sweden).

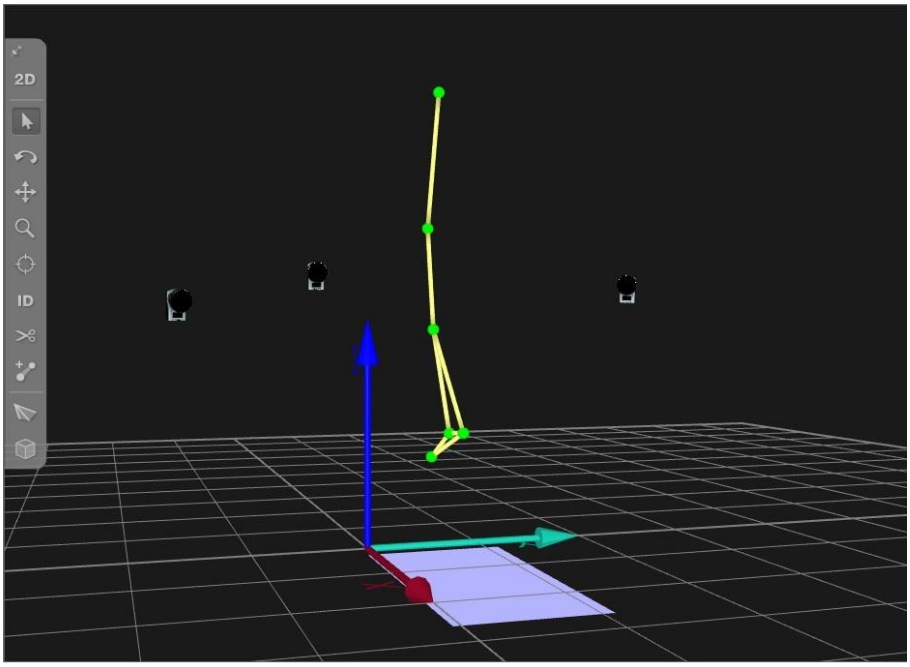

Figure 6: *Screen shot of the position data of a Maasai jumper during flight time in Qualisys. In the background are the setup of the three cameras used for kinematic recordings. The light blue area of the bottom picture represents the force plate, but is not to scale of the force plate used as only z-axis force was used for calibrations.*

4.3.3 Kinetic measures

The subjects tested at the Norwegian School of Sport Sciences jumped on a force plate (AMTI BP6001200, AMTI, Watertown, MA, USA) that was leveled to the ground. Data sampled in Tanzania was captured on a force plate built with a light-weight design (Biomekanikk AS, Oslo, Norway). As the force plate could not be lowered into the ground in Tanzania a lightweight frame of Styrofoam and plywood surrounded the plate (Figure 7). Sampling frequency of both force plates was set to 300 Hz. All recorded analog kinetic data was sent to a USB analog board (S/N 8161, Qualisys, AB, Gothenburg, Sweden), and converted to a digital signal. Digital data was exported to Qualisys Track Manager. To match the timeframes of the Oqus 400 cameras and the analog board a sync cable was connected between the two.


[redacted] [redacted] [redacted]

Figure 7: *One Maasai subject during flight phase of maximal repetitive jumping. On the bottom of the picture we see the light-weight force plate with the Styrofoam and plywood frame. On the subjects' right leg, we see the strapping of the US probe. Visible on the subjects' right side is also all marker placements.*

4.3.4 Ultrasonography

US images during repetitive jumping were sampled with LogicScan 128 with a 60mm linear array probe (scanning frequency: 7 MHz, LV7.5/60/96Z, Telemed, Lithuania). The probe was secured to the middle of GM with a custom-made cast strapped tightly around the shank to minimize any probe movement (Ishikawa & Komi, 2007; Ishikawa, Pakaslahti, & Komi, 2007; Lichtwark & Wilson, 2006; Sano et al., 2015) (Figure 7). US data was exported directly as an uncompressed AVI-file to Echo Wave II (3.4.2b5, Telemed, Lithuania). Capturing frequency was set to 64-82 images per second, but during saving as an AVI-file all frames ended up with a frequency of 81,45 Hz to 81,97 Hz. Image depth was set to 50 mm. A trigger cable was connected from the US to one of the Oqus 400 cameras and to Noraxon EMG to start the capturing of data at the same time.

4.3.5 Electromyography

Surface EMG was sampled from the left leg VL, GM, SOL and TA using bipolar surface electrodes (electrode material: Ag/AgCl, size: 45*22 mm, inter-electrode distance: 20 mm) (Neuroline 720 72000-S, Ambu, Ballerup, Denmark). The signals were sent from a wireless transmitter (Noraxon DTS EMG, Noraxon Inc., Scottsdale, AZ, USA) to a receiver (Noraxon TeleMyo DTS Desk Receiver, Noraxon Inc., Scottsdale, AZ, USA). The electrodes were placed according to SENIAM (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles) project (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000). Before electrode placement the skin was shaved and cleaned with alcohol. EMG was recorded from the contralateral (left) leg due to space constraints and risk of mechanical interference related to the quite large strapping of the US probe. Sampling frequency was set to 1500 Hz. EMG data was exported directly to Qualisys Track Manager.

4.4 Protocol

All Norwegian subjects met one time in the lab at the Norwegian School of Sport Sciences and all the Maasai one time in the field lab in Monduli Juu.

Anthropometric measures were taken before the jumping trial. During the jumping trial subjects were told to keep their hands at their hips and not pull their feet to the air. They began jumping repeatedly at a comfortable pace. The recording was not started until a controlled pace and a good rhythm was found. After one recording the subjects got an approximated two minutes break as data was saved. After 2-3 successful submaximal trials subjects were told to jump maximally, but still repeatedly. During these jumps, keeping balance and staying inside of the force plate was more challenging, and subjects were therefore told to look at a point or an item to better control their next landing. 2-3 successful maximal trials were captured. For both conditions subjects jumped for approximately 20 seconds during each trial, ten seconds were recorded and another ten seconds was used for finding balance and a controlled even pace. In addition to repetitive jumps the subjects also performed maximal jumps (countermovement jumps and squat jumps) and a maximal voluntary contraction test (*Appendix 2*).

4.5 Analysis and statistics

For each subject stance phase data from three cycles of repetitive jumping from 1-3 recordings have been averaged per subject in each condition (submaximal and maximal). Kinematic, kinetic, US and EMG data have been resampled to 200 points by fitting a spline in order to average data and run statistical analysis.

4.5.1 Anthropometic data

AT moment arm, foot length and toe length was calculated from pictures using ImageJ (National Institutes of Health, Maryland, USA). AT moment arm was considered as the mean value of the distance between the tip of the medial and lateral malleolus to the most posterior aspect of the AT (Scholz et al., 2008). Foot length was calculated from the distance between the most posterior point of the heel and the most anterior point of the hallux. Toe length was defined as the length of the hallux and were calculated from the distance between the most prominent aspect of the tip of the first metatarsal to the most anterior point of the hallux. Resting muscle architecture was calculated from US pictures using ImageJ (Figure 8). Both resting fascicle length and resting pennation angle was calculated three times from the same image for each subject and averaged. Lower leg circumference has been calculated as the percentage of largest measured circumference for each subject, before averaging for each group.

Figure 8: *US image of GM resting muscle architecture of a Maasai jumper. The picture includes lines along the path of the fascicle and aponeurosis in order to calculate resting pennation angle.*

4.5.2 Kinematic data

Position data was filtered with a Butterworth second-order low-pass filter with a cut-off frequency of 10Hz. Joint angles were calculated from the position of three markers for each joint. Ankle joint angle; lateral epicondyle, lateral malleolus, fifth metatarsal. Knee joint angle; trochanter major, lateral epicondyle, lateral malleolus. Hip joint angle; acromion, trochanter major, lateral epicondyle. The full length of GM MTU was calculated from a formula presented by Hawkins and Hull (1990):

$$L = C0 + C1\alpha + C2\beta + C3\beta^2 + C4\phi$$

$$GM\ MTU\ length\ = 0{,}900 + 0\alpha - 6{,}20E\text{-}4\beta + 0\beta^2 + 2{,}14E\text{-}3\phi$$

Where α, β, ϕ represents the hip-, knee-, and ankle joint angles at any given time.

4.5.3 Kinetic data

Force data was filtered with a Butterworth second-order low-pass filter (cut-off frequency 200 Hz). Initial ground contact and take-off from the force plate was used to determine the stance phase for all cycles of a recording.

4.5.4 Ultrasonography

US data was analyzed in MATLAB (R2015a, The MathWorks, Inc, Kista, Sweden) running UltraTrack (Farris & Lichtwark, 2016) (Figure 9). A single fascicle in the middle of the picture was identified and digitally tracked for each frame. To match the timestamps of force data to the timestamps of US data, force data was interpolated to match the US data. This was done in order to determine the initial ground contact and take-off times for US data. In addition to fascicle tracking the lower aponeurosis was tracked. This was done as fascicle tracking gives an angle of the fascicles relative to the absolute 0 degrees, and not the aponeurosis. For each recording the fascicle was tracked three times, and the aponeurosis once. This gave instantaneous fascicle length and its pennation angle relative to the lower aponeurosis. When calculating instantaneous GM tendinous tissues length, GM fascicle length multiplied by the cosine of its pennation angle was subtracted from GM MTU length (Fukunaga et al., 2001; Ishikawa, Komi, et al., 2005; Ishikawa, Niemela, & Komi, 2005; Lichtwark & Wilson, 2006; Sano et al., 2015):

$$GM\ TT\ length\ = GM\ MTU\ length - GM\ fascicle\ length * \cos(pennation\ angle)$$

Where GM TT length is the instantaneous length of GM tendinous tissues, GM MTU length is the instantaneous GM MTU length, GM fascicle length is the instantaneous GM fascicle length and pennation angle is the pennation angle of GM fascicles.

Figure 9: *Example of fascicle and aponeurosis tracking of one Maasai subject. The red area markes the region of interest, and is placed along the two aponeurosis, just around the muscle belly. Tracking of the fascicle and lower aponeurosis was done in separate trackings, but for informality is shown in the same image.*

4.5.5 Electromyography

EMG data was first band-pass filtered (5-500 Hz) before filtering with a Butterworth second-order low-pass filter. As the Maasai were unfamiliar with the concept of maximal voluntary contraction, no maximal EMG data could be reliably obtained. This limits the interpretation of EMG data to different patterns of pre-activation, braking and push-off. The pre-activation phase (PRE100ms) was defined from 100 ms before ground contact to ground contact. Braking phase (Braking) was defined from ground contact to peak length of GM MTU. Push-off phase (Push-off) was defined from peak GM MTU length to take-off. A root mean square envelope was applied to the signal before individually averaging the signal for each phase. The phases were divided by one another, and is presented as ratios of braking to PRE100ms and push-off to braking.

4.5.6 Statistics

For normally distributed variables the differences between the two groups were analyzed with an independent samples t-test. Normal distribution was tested with Shapiro-Wilk test. In addition, the data had to show a homogeneity of variance. This was tested with Levine’s test of Homogeneity of variance. Non-normally distributed data, and data that failed the Levine’s test, was analyzed for differences with a Mann-Whitney u-test. Statistical differences between submaximal and maximal repetitive jumping in each of the groups was tested by a related samples t-test. The non-parametric Wilcoxon signed ranks test was used for non-normal distribution between the difference of the variables. An alpha level of $p<0,05$ were used to determine statistical differences for all tests. Results in the article (Appendix 1) are presented as mean±SD (standard deviation). All statistical analysis was done in SPSS (International Business Machines, Armonk, New York, USA).

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Overview of figures

Appendix

1: Article

2: Full methods

3: Written consent form

4: Ethics approval

APPENDIX 1: Article

DIFFERENCES IN PROPERTIES OF THE TRICEPS SURAE MUSCLE-TENDON UNIT IN MAASAI JUMPERS AND CAUCASIAN CONTROLS DURING REPETITIVE JUMPING

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ABSTRACT

Purpose. The present study examined the anthropological features in ten Maasai males and investigated their muscle-tendon unit (MTU) function during repetitive jumping via fascicle behavior. The obtained data was compared to a group of six Caucasian males.

Methods. Ultrasonography, kinematics, kinetics and electromyography (EMG) data was recorded during two conditions; submaximal and maximal repetitive jumping.

Results. The Maasai jumpers had a longer Achilles tendon and foot than the Caucasian controls. During both conditions of repetitive jumping the Maasai jumpers had a lower jumping frequency, longer contact time, and a larger range of motion (ROM) of both the ankle, knee and hip than Caucasian controls. From submaximal to maximal condition frequency decreased, flight time was longer, and angular ROM increased for both groups. Fascicle behavior during ground contact was generally isometric for both groups, and the fascicle length of Maasai jumpers was shorter than that of Caucasian controls. The muscle-tendon unit (MTU) of both groups demonstrated a classical stretch-shortening cycle behavior during ground contact. The gastrocnemius medialis (GM) MTU length change and GM tendinous tissues length change was larger for the Maasai jumpers during both conditions compared to Caucasian controls. GM MTU length change was larger in maximal compared to submaximal condition for both groups. The EMG activation pattern of GM, SOL and VL was different between Maasai jumpers and Caucasian controls.

Conclusion. This study on Maasai jumpers revealed that they have optimized a jumping technique with a longer contact time and a larger ROM of the ankle, knee and hip than Caucasian controls. As a result of larger angular ROM the Maasai jumpers had a larger stretch of GM MTU and GM tendinous tissues. The longer AT and foot of the Maasai jumpers correlated weakly to jumping performance.

Keywords Ultrasonography • Muscle-tendon interaction • Repetitive jumping • Maasai • Tanzania

Abbreviations

AT	Achilles tendon
EMG	Surface electromyography
GM	Gastrocnemius medialis
MTU	Muscle-tendon unit
ROM	Range of motion
SOL	Soleus
SD	Standard deviation
SSC	Stretch-shortening cycle
TA	Tibialis anterior
TT	Tendinous tissues
US	Ultrasound
VL	Vastus lateralis

INTRODUCTION

The Maasai are specifically known for their rituals that involves chanting, dancing and repetitive jumps. The Maasai participating in the repetitive jumps of these rituals are young men known as warriors, usually between the age of 15-35 years old. Preliminary observations by Dirk Lund Christensen (Section of Global Health, University of Copenhagen, Copenhagen, Denmark) reports that many of the young men participating in these repetitive jumps move their body center of mass more than 50 cm. It seems that the participants of these rituals are great athletes, but accurate biomechanical analysis of their jumps, and the behavior of their muscle-tendon unit (MTU) during repetitive jumping has never been studied.

Jumping and repetitive jumping are natural, but complex human movements, where the human leg represents spring-like characteristics (Hobara, Kanosue, & Suzuki, 2007; Kuitunen, Ogiso, & Komi, 2011), and where the stretch-shortening cycle (SSC) of muscle-tendon function can be utilized effectively (Finni, Komi, & Lepola, 2001).

Less information is available on repetitive jumps than squat jumps, countermovement jumps and drop jumps (Lamontagne & Kennedy, 2013). During repetitive jumps the short ground contact time allows for small angular displacement about the ankle-, knee-, and hip joint, with the majority of the forces generated about the ankle- and knee joint (Finni et al., 2001). As the knee joint angular displacement is reduced the plantar flexor MTU provides more of the force needed to complete the movement compared to squat jumps and countermovement jumps.

Gastrocnemius medialis (GM) and the Achilles tendon (AT) are important structures of the plantarflexors for creating and transmitting force.

Anthropometrical features could explain the jumping performance of the Maasai. In geographical distance we need not look far to find other great athletes. The Kenyan endurance runners display some anthropological features of muscle and tendon in the lower leg that seems to enable them to utilize energy more efficiently during SSC movements (Sano et al., 2013; Sano et al., 2015).

When compared to physically active Caucasian males that was matched for height but not body mass Sano et al. (2013) found that Kenyan runners had a longer AT length at rest (26,4 cm vs. 19,7 cm) and a lower GM pennation angle (20 degrees vs. 21,8 degrees). Both groups performed maximal repetitive jumps. During these jumps the Kenyan runners had a shorter ground contact time, a longer flight time, and as a consequence of the two a 73,7±59,2 % greater jump height than the Caucasians (Sano et al., 2013). The seemingly efficient utilization of elastic energy during repetitive jumps was explained by a greater recoil from tendinous tissues (TT) despite a smaller range of length change in both fascicles and TT.

Sano et al. (2015) measured anthropometrics of Kenyan runners and compared the values to Japanese runners. Both groups were matched for height and weight, but the Kenyan runners had a longer lower leg (39,5 cm vs. 36,7 cm), longer AT moment arm (4,47 cm vs. 3,7 cm), longer GM MTU (43,4 cm vs. 35,3 cm) and longer GM TT (39,4 cm vs. 35,3 cm) (Sano et al., 2015). They also had shorter GM fascicles (4,8 cm vs. 5,7 cm) and greater GM pennation angle (20,6 degrees vs. 16,2 degrees). During running the Kenyan runners were able to utilize elastic energy more efficiently as the longer AT moment arm resulted in lower AT strain and GM muscle activation allowing fascicles to work more isometrically during ground contact (Sano et al., 2015).

The present study has two main aims.; to examine anthropometrical features in a group of Maasai jumpers and investigate MTU function during repetitive jumping, and to compare these data to those obtained in Caucasian controls.

From data obtained by Sano et al. (2013) and Sano et al. (2015) on Kenyan runners it is hypothesized that the Maasai jumpers has a long AT, a long AT moment arm, and that they display small fascicle length changes during the contact time of repetitive jumps.

MATERIAL AND METHODS

The following material and methods are part of a greater study on the biomechanics of Maasai people, for description of the full experimental trial see Appendix 2.

Subjects

Ten Maasai jumpers, three Norwegian elite jumpers and three physical active controls volunteered to participate in the study. The Maasai jumpers were recruited on site in the Monduli Juu region of Tanzania where a temporary field lab was set up. The Norwegian elite jumpers were all national elite level athletes in their respectable sports (basketball, volleyball and long jump). As the number of subjects in both the Norwegian elite jumpers group and controls are small, and statistic tests found no differences between the two groups for any variables, they have been merged to a Caucasian group. The anthropometric data of the two groups are presented in Table 1. Note that due to practical issues related to study terms and date of departure to Tanzania, the Caucasian group could not be matched for height and weight relative to the Maasai. All of the subjects signed a written informed consent form to take part in the study. The study was performed in accordance with the guidelines of the Declaration of Helsinki and was examined by relevant ethics committees in Norway and Tanzania.

Table 1 Age, weight, height, body mass index (BMI), foot length, toe length lower leg length (LLL), upper leg length (ULL), whole leg length (WLL), resting fascicle (rFa) length and resting pennation angle (rPa) for the Maasai and Caucasian subjects.

	Maasai (n=10)			**Caucasian (n=6)**			**P-value**
Age (yrs)	26,5	±	6,1	23,3	±	3,0	0,255
Height (cm)	172,0*	±	5,6	181,5	±	3,6	0,003
Weight (kg)	55,3*	±	7,4	82,7	±	10,5	<0,001
BMI (kg/m^2)	18,7*	±	2,0	25	±	2,7	<0,001
Foot length (cm)	26,41	±	1,21	25,93	±	1,05	0,431
Foot length/h (%)	15,4*	±	0,6	14,3	±	0,6	0,005
Toe length (cm)	7,03	±	0,81	6,74	±	0,3	0,588
Toe length/h (%)	4,1*	±	0,4	3,7	±	0,2	0,048
LLL (cm)	43,8	±	1,8	45,1	±	2,1	0,186
LLL/h (%)	25,6	±	0,7	25,0	±	0,9	0,199
ULL (cm)	41,6	±	1,5	42,6	±	3,1	0,384
ULL/h (%)	24,2	±	0,8	23,5	±	1,6	0,492
WLL (cm)	85,4	±	3,1	87,7	±	5,0	0,447
WLL/h (%)	49,6	±	1,4	48,3	±	1,9	0,147
rFa (cm)	5,11*	±	0,80	6,20	±	0,55	0,011
rPa (°)	20,2	±	3,0	20,1	±	3,3	0,956

* significant statistical difference between Maasai and Caucasian groups ($p<0,05$).

Protocol

Overview

Age was first noted and height and bodyweight was measured. Pictures were taken laterally and medially of the foot while standing on a custom made measurement board to enable assessment of AT moment arm, foot length and toe length. Free AT length, AT aponeurosis length and GM muscle architecture was measured with ultrasound (US). Lower leg length, upper leg length and regional distribution of lower leg mass were measured before subjects performed repetitive jumps at two different intensities. Subject were instructed to keep their hands on the hip at all times, and not pull their feet up during the flight phase of the jump. For the first set of repetitive jumps they were instructed to jump at a preferred pace. For the second set of repetitive jumps they were instructed to perform maximal repetitive jumps. During the maximal repetitive jumps subjects were told to focus on a point in order to keep balance. The recording was not started until a controlled pace and a good rhythm was found. Under both conditions each trial lasted approximately 20 seconds, with recording accounting for half of that time. The other ten seconds was used for finding balance and a controlled even pace. In between recordings subjects got approximately two minutes of rest while data was saved. 2-3 recordings were sampled from each of the two different conditions.

Measured parameters

Anthropometrics. Height, bodyweight, leg length, lower leg length, upper leg length, and distribution of lower leg volume was measured with the subjects in a standing position. Lower leg circumference was measured at nine different lengths of the lower leg.

Free AT length and AT aponeurosis length was measured with the subjects lying in a supine position on a bench reaching mid-way to their shank. An US system, LogicScan 128 (EXT-1Z, Telemed, Lithuania), with a linear 60 mm transducer (scanning frequency: 12 MHz, HL9.0/60/128Z-2, Telemed, Lithuania), was used to find AT insertion on calcaneus, the first insertion of Soleus (SOL) on AT and GM insertion on AT. The distance from the insertion on calcaneus to the most distal insertion of SOL is considered the free AT length, and the distance from the distal insertion point of SOL to the insertion of GM on the aponeurosis is considered AT aponeurosis length. Both distances were measured externally (Stafilidis & Arampatzis, 2007). The sum of these two distances was defined as the AT length. Us images of GM resting muscle architecture was acquired in the same supine position at the lower 30 % of GM muscle length. AT moment arm, foot length and toe length was calculated from pictures of the foot taken in a standing position as described by Scholz, Bobbert, van Soest, Clark, and van Heerden (2008) (Figure 1).

Figure 1 Zoomed-in lateral and medial view of the right foot of one Maasai subject on the custom built measurement board. From these pictures AT moment arm was calculated. Foot length was calculated form medial view pictures with the tip of the toe at center of image. Toe length was calculated from medial view pictures with the hallux at center of image. The images were taken from distance with different centers to minimize distortion.

Kinetics. For technical reasons (limitation related to shipping weight) two different platforms were used. A lightweight force plate (Biomekanikk AS, Oslo, Norway) (Figure 2) was used for the Maasai subjects while an AMTI (BP6001200, AMTI, Watertown, MA, USA) force plate was used testing the Caucasian subjects. Sampling frequency was set to 300 Hz. Analog force data was sent to a USB analog board (S/N 8161, Qualisys, AB, Gothenburg, Sweden) and converted to a digital signal. Force data was filtered with a Butterworth second order low-pass filter with a cut-off frequency of 200 Hz.

Kinematics. Two-dimensional kinematic data were recorded with three retro-reflective cameras (Oqus 400, Qualisys, AB, Gothenburg, Sweden) at 300 Hz. 12 mm reflective markers were placed on six bony anatomical landmarks (acromion, trochanter major, the lateral epicondyle, tip of the lateral malleolus, on the approximate insertion of AT on calcaneus, and the fifth metatarsal head) (Figure 2). Position data was filtered with a Butterworth second-order low-pass filter with a cut-off frequency of 10 Hz.

Ultrasonography. Ultrasonography was applied to measure GM fascicle length and pennation angle during the repetitive jumps. The US probe was positioned on the middle of the right leg GM, and secured to the leg using a custom-made cast and a bandage wrapped tightly around the shank to minimize any probe movement (Ishikawa & Komi, 2007; Ishikawa, Pakaslahti, & Komi, 2007; Lichtwark & Wilson, 2006; Sano et al., 2015) (Figure 2). A LogicScan 128 was connected to a 60 mm linear array prove probe (scanning frequency: 7 MHz, LV7.5/60/96Z, Telemed, Lithuania). Depending on the dynamic range of the image the frequency of the US was between 72-84 images per second, with a 50 mm image depth. US data was saved as an uncompressed AVI-file from Echo Wave II (3.4.2b5, Telemed, Lithuania).

Figure 2 One Maasai subject during flight phase of maximal repetitive jumping. On the bottom of the picture we see the light-weight force plate with the Styrofoam and plywood frame. On the subjects' right leg, we see the strapping of the US probe. Visible on the subjects' right side is also all marker placements.

Surface electromyography. Surface electromyography (EMG) was recorded from the left leg GM, SOL, tibialis anterior (TA) and vastus lateralis (VL) using eight bipolar surface electrodes (electrode material: Ag/AgCl, size: 45*22 mm, inter-electrode distance: 20 mm) (Neuroline 720 72000-S, Ambu, Ballerup, Denmark) connected to a Noraxon DTS EMG wireless transmitter (Noraxon Inc., Scottsdale, AZ, USA). The signals were transferred wireless to Noraxon TeleMye DTS Desk Reciever (Noraxon Inc., Scottsdale, AZ, USA). The placement of electrodes followed the SENIAM guidelines (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000). Before electrode placement the skin was shaved and cleaned with alcohol. EMG was recorded from the contralateral (left) leg due to space constraints and to avoid any disturbance in the signal related to the quite large strapping of the US probe. Sampling frequency was set to 1500 Hz. EMG data was band-pass filtered (5-500 Hz) before being filtered with a Butterworth second-order low-pass filter.

The US apparatus triggered the data collection, and was connected to one of the Oqus 400 cameras and Noraxon EMG by cable. The same camera connected with the trigger cable, was also connected to the USB analog board by a sync cable. This ensured that kinetic, kinematic, US and EMG data sampling was started at the same time. Kinetic, kinematic and EMG data was saved in Qualisys Track Manager (Qualisys, AB, Gothenburg, Sweden)

Analyses

Anthropometry and tendon measurements. AT moment arm, foot length and toe length was calculated from pictures using ImageJ (National Institutes of Health, Maryland, USA). AT moment arm was considered as the mean value of the horizontal distance between the tip of the medial and lateral malleolus to the most posterior aspect of the AT. Foot length was calculated from the distance between the most posterior point of the heel and the most anterior point of the hallux. Toe length, defined as the length of the hallux, was calculated from the distance between the most prominent aspect of the tip of the first metatarsal to the most anterior point of the hallux.

Resting muscle architecture was calculated from US pictures using ImageJ. Both resting fascicle length and resting pennation angle was calculated three times for each subject and averaged.

Lower leg circumference has been calculated as the percentage of largest measured circumference for each subject before averaging for each group (Figure 4).

Repetitive jumping. For each subject stance phase data from three cycles of repetitive jumping from 1-3 recordings were averaged per in each condition (submaximal and maximal). Each stance phase was determined by initial ground contact and take-off from the force plate. In order to average data and run statistical analysis, kinematics, kinetics, US and EMG have been resampled to 200 points by fitting a spline.

US data was analyzed in MATLAB (R2015a, The MathWorks, Inc, Kista, Sweden) running UltraTrack (Farris & Lichtwark, 2016). A single fascicle in the middle of the picture, or the lower aponeurosis, was identified and digitally tracked for each frame (Figure 3). For each recording the fascicle was recorded three times, and the aponeurosis once. This gave instantaneous fascicle length and its pennation angle relative to the lower aponeurosis.

Figure 3 Example of fascicle and aponeurosis tracking of one Maasai subject. The red area markes the region of interest, and is placed along the two aponeurosis, just around the muscle belly. Tracking of the fascicle and lower aponeurosis was done in separate trackings, but for informality is shown in the same image.

Joint angles were calculated form the position of three markers for each joint, ankle joint angle (lateral epicondyle, lateral malleolus, fifth metatarsal), knee joint angle (trochanter major, lateral epicondyle, lateral malleolus) and hip joint angle (acromion, trochanter major, lateral epicondyle). GM MTU length was calculated form the formula presented by Hawkins and Hull (1990):

$$L = C0 + C1\alpha + C2\beta + C3\beta^2 + C4\phi$$

$$GM\ MTU\ length = 0{,}900 + 0\alpha - 6{,}20E\text{-}4\beta + 0\beta^2 + 2{,}14E\text{-}3\phi$$

Where α, β, ϕ represents the hip-, knee-, and ankle joint angles at any given time.

Instantaneous length of GM TT was calculated by subtracting GM fascicle length multiplied by the cosine of its pennation angle (Sano et al., 2015):

$$GM\ TT\ length = GM\ MTU\ length - GM\ fascicle\ length * \cos(pennation\ angle)$$

As the Maasai were unfamiliar with the concept of maximal voluntary contraction, no maximal EMG data could be reliably obtained. This limits the interpretation of EMG data to different patterns of pre-activation, braking and push off. The pre-activation phase (PRE100ms) was defined from 100 ms before ground contact to ground contact. Braking phase (Braking) was defined from ground contact to peak GM MTU length. Push-off phase (Push-off) was defined from peak GM MTU length to take-off. A root mean square envelope was applied to the signal before individually averaging the signal for each phase. To calculate EMG ratios, the averaged phases were divided by one another and is presented as ratios of braking to PRE100ms and push-off to braking.

Statistics

Statistical differences between the groups were tested with an independent samples t-test or Mann-Whitney u-test. Variables that did not pass Shapiro-Wilks test for normal distribution and Levine's test of Homogeneity of variance were analyzed with the non-parametric Mann-Whitney u-test. Statistical differences between submaximal and maximal repetitive jumping in each of the groups was tested by a related samples t-test. The non-parametric Wilcoxon signed ranks test was used for non-normal distribution between the difference of the variables. An alpha level of $p<0,05$ were used to determine statistical differences for all tests. Results are presented as mean±standard deviation (SD). Unless stated otherwise the 200 points during stance phase was averaged for each group before statistical analysis. All statistical analysis was done in SPSS (International Business Machines, Armonk, New York, USA)

RESULTS

Anthropometric data. The anthropometric analyses showed that the two groups were similar in age, and in absolute values of foot length, toe length, lower leg length, upper leg length and whole leg length. Related to body height the Maasai had a greater foot and toe length. No difference was found for lower leg length, even related to body height. The resting fascicle length was shorter for the Maasai group, and no difference was found in resting pennation angle. Compared to the Maasai subjects the Caucasians were heavier and taller, but due to the large weight difference their Body Mass Index also was significantly larger (Table 1).

Tendon measurements and AT moment arm. There were no significant differences in free AT length or the ratio of free AT length to height between the two groups. A significant statistical difference in AT aponeurosis length and AT aponeurosis length to height was found with the Maasai having both a longer AT aponeurosis length and a higher percentage of AT aponeurosis to height. A statistical significant difference was found for both AT length and AT length to height with the Maasai having a longer AT and a higher percentage of AT length to height. No statistical difference was found for AT moment arm (Table 2).

Table 2 Tendon measurements and calculated AT moment arm of the Maasai and Caucasian subjects. Aponeurosis (apo), lower leg length (LLL).

	Maasai (n=10)			**Caucasian (n=6)**			**P-value**
Free AT length(cm)	5,2	±	1,2	6,7	±	2,3	0,098
Free AT length /height (%)	3,0	±	0,7	3,7	±	1,3	0,185
AT apo length(cm)	18,1*	±	1,7	14,8	±	2,7	0,017
AT apo length/height (%)	10,6*	±	1,0	8,1	±	1,6	0,003
AT length (cm)	23,3*	±	1,2	21,5	±	1,7	0,029
AT length/height (%)	13,6*	±	0,8	11,8	±	0,8	0,001
AT moment arm (cm)	4,79	±	0,21	4,78	±	0,09	0,664
AT moment arm/height (%)	2,8	±	0,1	2,6	±	0,2	0,051

* significant statistical difference between Maasai and Caucasian groups (p<0,05).

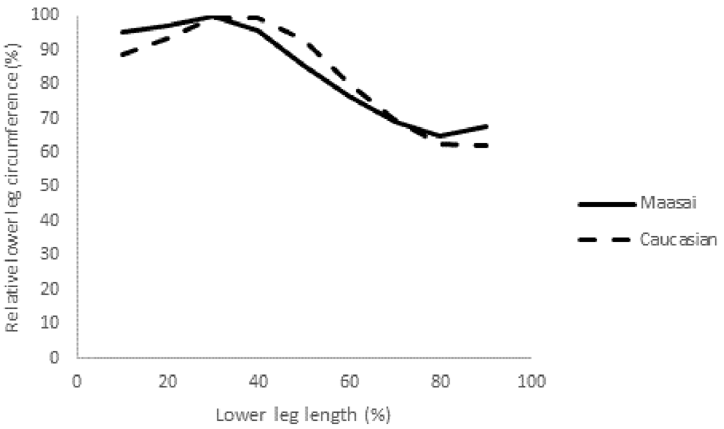

Figure 4 Relative lower leg circumference of the Maasai and Caucasian group. 100 % is the largest of the nine measurements for each subject. Data has been averaged for each group.

Repetitive jumping. The Maasai group and the Caucasian group jump at different frequencies. When dividing the jump cycle into contact time and flight time it is clear that the higher jumping frequency of the Caucasians during both submaximal and maximal repetitive jumping comes from a shorter contact time, and not a shorter flight time (Table 3). No difference of contact time was found for the two conditions for either the Maasai group (p=0,333) or the Caucasian group (p=0,205), but there was a difference in jumping frequency

(Maasai: p<0,001) and Caucasian: p=0,001) and flight time (Maasai: p<0,001 and Caucasian: p=0,001).

Table 3 Jump frequency, time per jump, contact time and flight time for both the Maasai and Caucasian subjects during submaximal and maximal repetitive jumps.

Submaximal	**Maasai (n=10)**			**Caucasian (n=6)**			**P-value**
Jump frequency (Hz)	1,63*	±	0,11	2,24	±	0,39	0,007
Time per jump (s)	0,62*	±	0,05	0,46	±	0,09	<0,001
Contact time (s)	0,28*	±	0,02	0,18	±	0,02	<0,001
Flight time (s)	0,34	±	0,04	0,28	±	0,10	0,140
Maximal							
Jump frequency (Hz)	1,26*	±	0,10	1,52	±	0,14	0,001
Time per jump (s)	0,80*	±	0,06	0,66	±	0,06	0,001
Contact time (s)	0,31*	±	0,08	0,18	±	0,02	0,002
Flight time (s)	0,49	±	0,07	0,48	±	0,07	0,883

* significant statistical difference between Maasai and Caucasian groups (p<0,05).

The displacement of ankle, knee and hip angle during ground contact was as expected during a SSC movement for both submaximal and maximal repetitive jumping (Figure 5). The ankle was dorsiflexed during braking, and plantarflexed during the push-off. Both the knee and the hip was first flexed during ground contact before extending during push-off. The Maasai jumpers had a larger range of motion (ROM) for the ankle joint, and a larger ROM of both the knee and the hip compared to the Caucasian group (Figure 5). There was a difference in the joint angle of the knee and the hip at initial ground contact between the two groups during the submaximal condition, but even with that taken into account the average knee and hip joint angle was different between the groups (Knee: p=0,083 and hip: p=0,129).

Fascicle behavior seems to be generally isometric with both groups showing small to no change in fascicle length (Figure 6). In absolute values the Maasai group had a shorter fascicle length during submaximal repetitive jumping (Maasai: 3,32±0,54 cm vs. Caucasian: 4,77±0,91 cm, p=0,001) and greater pennation angle (Maasai: 29,1±5,9° vs. Caucasian: 22,6±4,2°, p=0,031). During maximal repetitive jumping the Maasai group had a shorter absolute fascicle length (Maasai: 3,58±0,51 cm vs. Caucasian: 4,69±0,97 cm, p=0,030), while no difference was found in absolute pennation angle (Maasai: 27,0±5,6° vs. Caucasian: 23,0±4,0°, p=0,149). No differences were found between the two groups in any condition when normalizing fascicle length and pennation angle to resting fascicle length and resting pennation angle (Figure 6).

The MTU of both groups demonstrated a classical stretch-shortening behavior during ground contact under both conditions (Figure 7). The maximal length of the MTU was reached at 50±0,8 %. The length of the MTU and TT was, under both conditions, longer for the Maasai group when normalizing for standing MTU length (Figure 7). In absolute values no difference was found for either MTU length (Submaximal: Maasai: 48,5±2,5 cm vs. Caucasian: 48,6±2,1 cm, p=0,943, Maximal: Maasai: 49,0±2,5 cm vs. Caucasian: 49,4±2,1 cm, p=0,794) or TT length (Submaximal: Maasai: 45,6±2,4 cm vs. Caucasian: 44,1±1,7 cm, p=0,197, Maximal: Maasai: 45,8±2,7 cm vs. Caucasian: 45,0±2,0 cm, p=0,538).

Table 4 Measured and calculated averaged variables during submaximal repetitive jumping for Maasai and Caucasian. Fascicle (Fa), pennation angle (Pa), resting fascicle (rFa), resting pennation angle (rPa), standing MTU (sMTU).

	Maasai (n=10)			**Caucasian (n=6)**			**P-value**
Ankle angle (°)	83,9*	±	5,7	96,2	±	4,6	0,001
Knee angle (°)	138,5*	±	4,8	160,7	±	9,7	0,001
Hip angle (°)	156,2*	±	7,0	171,1	±	6,0	0,001
Fa length/rFa length	0,66	±	0,10	0,77	±	0,12	0,064
Pa/rPa	1,45	±	0,31	1,15	±	0,33	0,088
MTU length/sMTU length	1,15*	±	0,02	1,11	±	0,02	0,005
TT length/sMTU length	1,08*	±	0,02	1,01	±	0,04	<0,001

* significant statistical difference between Maasai and Caucasian groups (p<0,05).

Table 5 Measured and calculated variables during maximal repetitive jumping for Maasai and Caucasian. Fascicle (Fa), pennation angle (Pa), resting fascicle (rFa), resting pennation angle (rPa), standing MTU (sMTU).

	Maasai (n=10)			**Caucasian (n=6)**			**P-value**
Ankle angle (°)	80,3*	±	4,7	91,5	±	4,1	<0,001
Knee angle (°)	129,6*	±	6,6	149,4	±	5,2	<0,001
Hip angle (°)	141,6*	±	12,5	160,3	±	7,3	0,005
Fa length/rFa length	0,71	±	0,13	0,75	±	0,13	0,548
Pa/rPa	1,34	±	0,29	1,17	±	0,36	0,306
MTU length/sMTU length	1,17*	±	0,02	1,13	±	0,02	0,007
TT length/sMTU length	1,09*	±	0,02	1,03	±	0,04	0,002

* significant statistical difference between Maasai and Caucasian groups (p<0,05).

As the behavior of joint displacement, fascicles and MTU are the same in both jumping conditions, the variables was tested for differences of average values during ground contact. There was a difference in average ankle, knee and hip joint angle, and MTU length relative to standing MTU length between submaximal and maximal jumping for both groups (Maasai: $p \leq 0{,}002$ and Caucasian: $p \leq 0{,}004$). For TT relative to standing MTU length behavior there was no difference in average length for the Maasai group (p=0,39) but a difference for the Caucasian group (p=0,005). On the other hand, average fascicle to resting fascicle length that was not the same for Maasai (p=0,047) but it was for Caucasians (p=0,6).

Both in submaximal and maximal repetitive jumping a difference in EMG ratio of braking to PRE100ms (Braking/PRE100ms) between the two groups were only present in VL (p=0,006 and p=0,043, respectively). In EMG ratio of push-off to braking (Push-off/Braking) a difference between the two groups were found under both conditions for GM (p=0,007 and p=0,027) and SOL (p=0,005 and p=0,012) (Figure 8). Overall there was a higher EMG activation during braking than PRE100ms, with the highest ratio in SOL and VL, and a higher activation during braking than push-off under both conditions and for both groups.

In the following discussion the terms fascicle length, pennation angle, MTU and TT refers to the average relative values found in Table 4 and Table 5. If absolute values or phases of contact time as discussed this will be clarified.

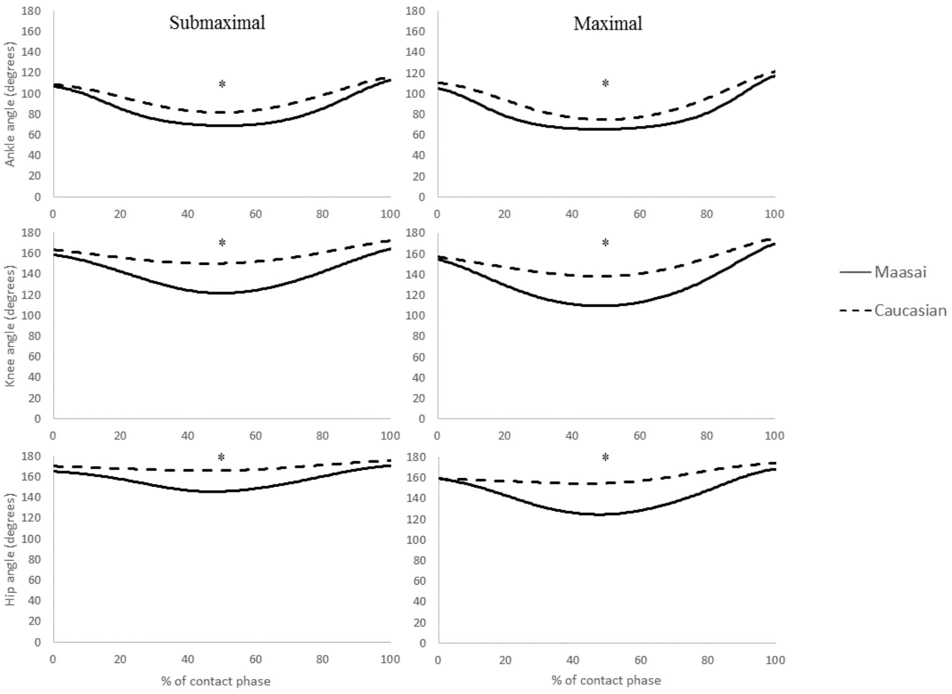

Figure 5 Submaximal repetitive jumping (left) and maximal repetitive jumping (right) ankle- knee- and hip joint angle of the Maasai and Caucasian group during contact phase. * significant statistical difference between Maasai and Caucasian groups (p<0,05).

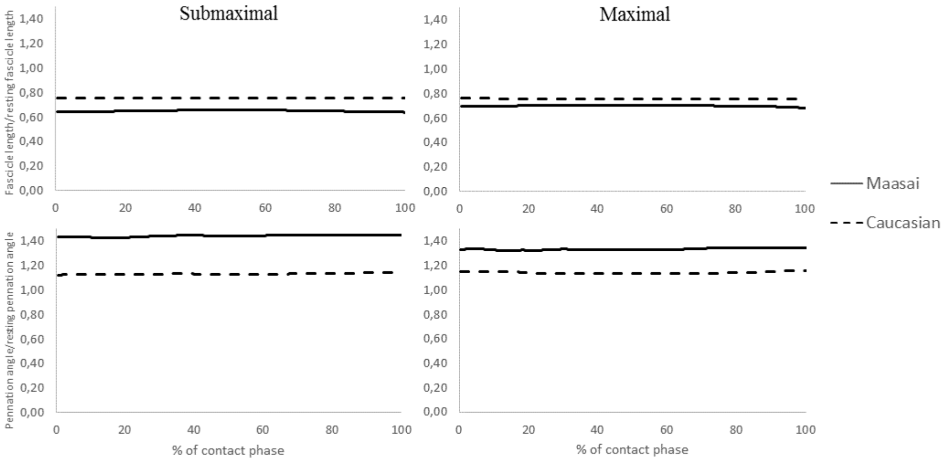

Figure 6 Submaximal repetitive jumping (left) and maximal repetitive jumping (right) ratio of fascicle length/resting fascicle length and pennation angle/resting pennation angle for the Maasai and Caucasian group during contact phase. * significant statistical difference between Maasai and Caucasian groups (p<0,05).

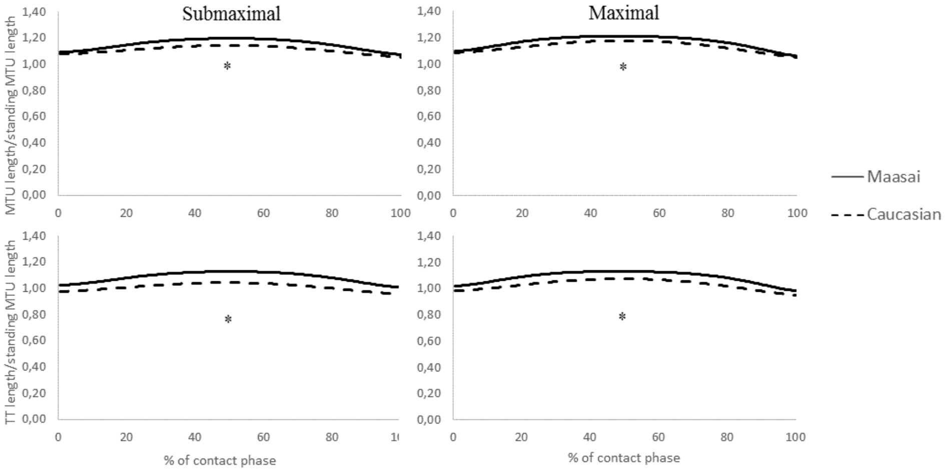

Figure 7 Submaximal repetitive jumping (left) and maximal repetitive jumping (right) MTU length/standing MTU length and TT length/standing MTU length for the Maasai and Caucasian group during contact time. * significant statistical difference between Maasai and Caucasian groups (p<0,05).

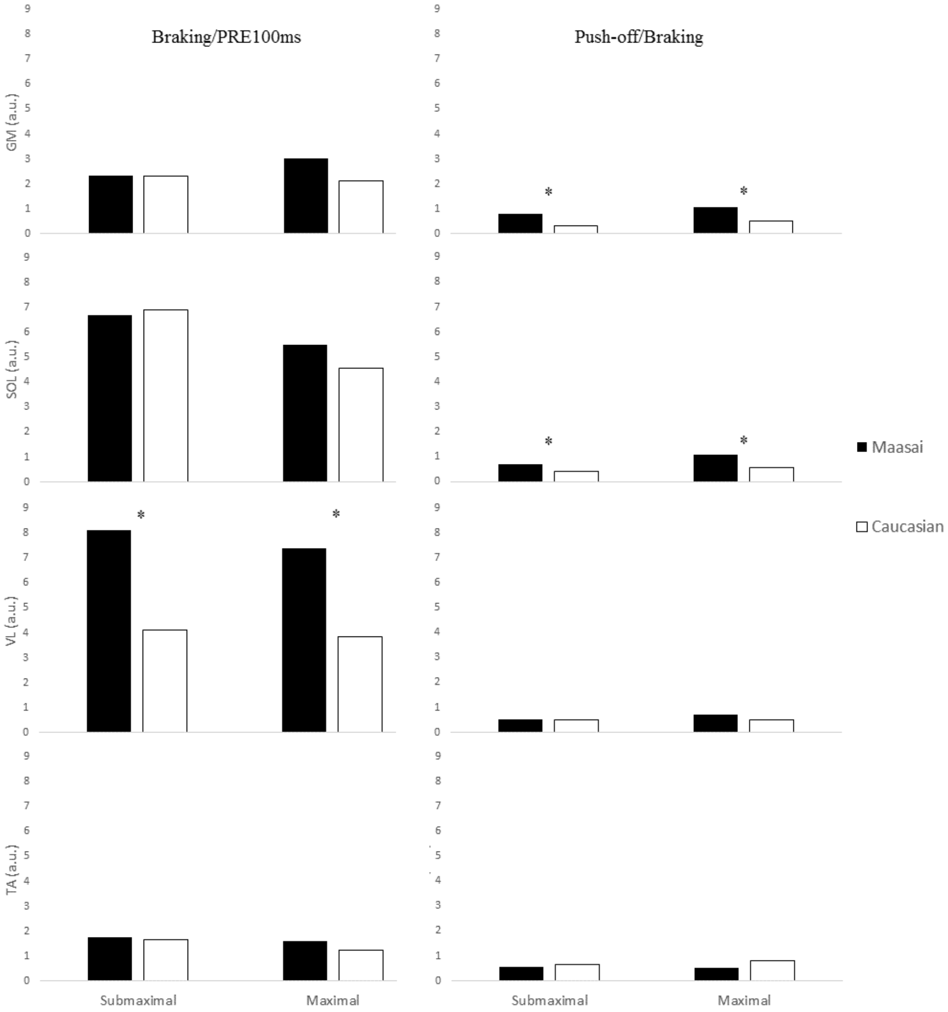

Figure 8 EMG ratios between braking/PRE100ms and push-off/braking for Maasai jumpers and Caucasian controls. The ratios were calculated from braking to PRE100ms (Braking/PRE100ms) and push-off to braking (Push-off/Braking). * significant statistical difference between Maasai and Caucasian groups (p<0,05).

DISCUSSION

The present study examines the anthropometric features of a group of Maasai jumpers and MTU function during both submaximal and maximal repetitive jumping. Research of this kind has never been conducted on a group of Maasai jumpers. Interpretation of these results will try to give an insight to anthropometric features and MTU function of the Maasai during repetitive jumping.

Main findings. It was hypothesized that the Maasai jumpers has a long AT, a long AT moment arm, and that they display small fascicle length changes during contact time of repetitive jumps. Confirming the first hypothesis Maasai jumpers had a longer AT than Caucasian controls, both absolute and relative to height (Table 2). Maasai jumpers also displayed small fascicle length changes during contact time of both submaximal and maximal repetitive jumping (Figure 6). The fascicle behavior was not different from that of Caucasian controls. The calculated AT moment arm was the same for Maasai jumpers and Caucasian controls (Table 2). The Maasai subjects jumped at a lower frequency and with a longer contact time than Caucasian subjects (Table 3). There was a larger stretch of both the MTU and TT during ground contact for the Maasai jumpers compared to Caucasians under both conditions (Figure 7).

Jumping performance and joint angles. During both conditions of repetitive jumping the two groups chose different strategies. There was a difference in jump frequency, and therefore time between each jump cycle, only relating to contact time during the stance phase (Table 3). As there is no difference in ground contact time with increased frequency, it looks like the two groups have a preferred contact time, as observed by Moritani, Oddsson, and Thorstensson (1991) where contact time did not change from submaximal 2 Hz repetitive jumping to maximal. The contact time is shorter for the Caucasian group than the Maasai, and is likely to explain the larger ROM of the ankle, knee and hip joint of the Maasai jumpers during ground contact (Figure 5). It is easy to conclude that the larger contact time allows for a larger ROM, but it could also be that joint ROM is optimized for storage and release of elastic energy from tendons, and in that way defines the contact time.

The flight time can be used to measure jump height ($\left(\frac{1}{8}\right) gt^2$). As there is no significant difference in flight time between the two groups for the two conditions, the resulting jump height is not different. With this in mind, the flight time of the Maasai jumpers tends to be higher during submaximal (22,9 %) and maximal (1,1 %) condition.

Muscle behavior. For both groups, and during both conditions the behavior of the fascicles was rather isometric (Figure 6), similar to behavior of the Kenyan runners during maximal repetitive jumping (Sano et al., 2013). On the contrary this is not the fascicle behavior Sano et al. (2013) observed from Caucasian males that contracted their fascicles to a greater extent. In SSC movements like running or jumping fascicles work at the ascending limb of the force-length relationship (Ishikawa et al., 2007). The fascicles of the Maasai jumpers worked at a shorter length relative to the resting fascicle length than the Caucasians. A short fascicle length during SSC movement is believed to favor storage of elastic energy in TT (Ishikawa et al., 2007). From submaximal to maximal condition the Maasai jumpers had statistically significant longer fascicles (Figure 6). The effects of this length change is not easy to measure, but during short-contact SSC movements it has been suggested that the efficiency of muscle contraction in plantarflexors is optimized for TT to store and release elastic energy (Hof, 2003).

EMG activation of GM and SOL was higher during the braking phase than the pre-activation as seen by the high ratio of braking to PRE100ms (Figure 8). The activation of the following push-off phase was significantly higher for the Maasai group compared to the controls for both GM and SOL (Figure 8). A higher EMG of GM and SOL during push-off is considered to correlate with a higher shortening velocity (Sano et al., 2015). The higher activation of GM and SOL during push-off could be a strategy to keep fascicles from stretching during the push-off. On the other hand, it is possible that the relative amount of activation during push-off was similar between groups, but that the Maasai jumpers used less activation during braking to enable a larger ROM of the ankle and in that way larger storage of elastic energy.

In ratio of braking to PRE100ms the Maasai jumpers had a significantly larger VL activation than the Caucasian controls (Figure 8). The higher activation of VL for the Maasai jumpers in the braking phase indicates that the two groups has a different contribution of knee extensor muscles in repetitive jumps. As no inverse dynamics has been calculated to determine joint moments the contribution of VL to jumping performance of the Maasai subjects is not clear. As VL has a greater muscle volume, longer fibers and a shorter tendon than GM it would be advantageous for doing mechanical work (Kuitunen et al., 2011). Combining the storage and release of elastic energy from the AT with the mechanical work of knee extensors would be advantageous for greater jumping heights.

Tendon behavior. The Maasai jumpers had a longer AT than the Caucasian controls. This was explained by a longer AT aponeurosis length, and not from the free tendon length. The length of the AT showed no correlation to maximal jumping performance across all subjects (r=0) even when adjusting for height (r=0,08). For Caucasian subjects alone there was a moderate correlation of AT length to maximal jumping performance (r=0,41). The correlation of AT length to jumping performance showed a weak negative relationship for the Maasai jumpers (r=0,29). As AT length alone does not seem to have any impact on the maximal jumping performance, the muscle-tendon interaction and the mechanical properties of the tendon seems to be of greater importance to maximal jumping performance.

As the fascicles work isometrically and pennation angle does not change during ground contact for both groups, and during both conditions, the change in length of contractile tissues $(fascicle\ length * \cos(pennation\ angle))$ is as a result rather constant. Any changes of MTU length observed is almost entirely related to stretch of TT. During both conditions the Maasai had a larger stretch of the MTU, and a larger stretch of TT (Figure 7).

When looking at the percentage of TT stretch from standing length to peak TT length during ground contact, values between 5,0 % and 8,1 % are reported during submaximal and maximal SSC movements (Ishikawa & Komi, 2007; Lichtwark & Wilson, 2005, 2006; Sano et al., 2013). Common for these values is that the resting muscle architecture are measured while standing. As the resting muscle architecture of the Maasai jumpers and Caucasian controls of this study are measured from a supine, non-bearing condition, the fascicles may be less stretched and perhaps consequently the pennation angle is larger. This influences the calculations of contractile tissues length, and in that way the TT length. The range of absolute TT length stretch ranged from 4,7 % to 8,4 % from submaximal to maximal condition for the Caucasian group. This is within the previously reported values, but as calculations are different it cannot be directly compared. Even more interesting are the same values for the Maasai jumpers of 14,2 % and 23,6 %. The interpretation of this is not easy, as there are no reports found for change in fascicle length and pennation angle from non-bearing conditions to standing. These values would anyway not be appropriate for a sub-population like the Maasai.

In light of the larger ankle ROM during ground contact for the Maasai jumpers, and their longer AT a larger stretch of TT is expected. The Kenyan runners had stiffer tendons than Caucasian controls resulting in a smaller change of TT length from standing to peak TT during maximal repetitive jumping (Sano et al., 2013). It seems to be the opposite for Maasai jumpers that show a larger percentage of TT stretch, and therefore might have more compliant tendons.

It could be that the change in resting fascicle length is large from non-bearing condition to standing. As the stretch of absolute length of TT is larger for the Maasai jumpers than the Caucasians it could be that their tendon is less stiff. while still seemingly utilizing elastic energy from tissues effectively.

When looking at anthropometrics the Maasai had longer feet and a longer toe than the Caucasians when adjusting for body height (Table 1). The foot length measured are similar to the measurements by Choi, Suh, and Seo (2014) for Maasais of the same age, weight and height. No difference was found for AT moment arm in absolute or relative scale, but as the foot functions as a lever about the same joint as the AT moment arm, a difference in foot length will affect the moment about the joint. It has been suggested that the length of the AT moment arm has a greater influence on performance than the length of AT itself (Scholz et al., 2008). The effect of AT moment arm on jumping performance showed no correlation to maximal jumping performance (r=0,08) even when adjusting for height (r=0). As there is no statistical difference in either AT moment arm or flight time between the two groups this is as expected. For foot length only a weak correlation to flight time was found during maximal repetitive jumping (r=0,23) and when correcting for height differences the relationship was even weaker (r=0,14). The weak relationship of AT moment arm and foot length to jump performance further emphasize the importance of muscle-tendon interaction in repetitive jumping performance.

Limitations. The most obvious limitation to this study is the fact that the Caucasians could not be matched for height and weight to the Maasai jumpers. In addition, the Caucasian control group is small and consisting of both active controls and jumping athletes. With a matched control group interpretation of data would be easier as they would not need to be normalized. Interpretation of MTU, fascicle and TT behavior has been limited to average values, as movement patterns were the same between both groups. By dividing MTU, fascicle and TT behavior into different phases of ground contact (braking, push-off) one would further improve the understanding of MTU function of the Maasai jumpers during repetitive jumps.

The difference in jumping performance between the two groups has been calculated from flight time. A better measure of performance could be calculated from ground reaction forces. Ground reaction forces would also have improved the interpretation of joint ROM, and if forces are captured in all three axis ground reaction forces could be used to calculate joint moments. The interpretation of EMG activation was limited to ratios of pre-activation, braking activation and push-off activation. To better understand the magnitude of EMG activation the data could be improved by normalizing the EMG signal to the peak activation during maximal repetitive jumps. To be able to calculate percentage of TT stretch from standing to peak length change during jumping fascicle length could have been measured in a standing position as well as resting fascicle length.

Conclusion. Knowing the limitations of the present study, and the fact that this is the first study of its kind on Maasai jumpers, conclusions should be drawn with care. From the present results it is concluded that the Maasai jumpers has optimized a jumping technique with longer contact time and larger ROM of the ankle, knee and hip than the Caucasian controls. The result of the larger angular ROM of the Maasai jumpers was a larger stretch of the MTU that in light of fascicle-tendon interaction resulted in a larger stretch of TT compared to Caucasian controls. The longer AT and foot of the Maasai jumpers correlated weakly with jumping performance.

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Full experimental trial

All Caucasian subjects met one time in the lab at the Norwegian School of Sport Sciences and all the Maasai one time in the field lab in Monduli Juu.

Anthropometric measures were taken before the maximal isometric plantar flexion and the jumping trials. First, measures of height and weight was taken, and age was reported. As the Maasai don't know their age, their reported age is only an approximation. Pictures was taken of the foot standing on a measure board before free AT length and AT aponeurosis length, along with resting muscle architecture and muscle thickness of GM and VL, was measured with an ultrasound system, LogicScan 128 (EXT-1Z, Telemed, Lithuania), with a linear 60mm transducer (scanning frequency: 12MHz, HL9.0/60/128Z-2, Telemed, Lithuania) from a supine position. Lower leg length, upper leg length and distribution of lower leg mass was measured with a measuring tape from a standing position.

The following order of the three tests was not the same in Norway and Tanzania. Caucasian subjects did maximal jumps, repetitive jumps and maximal isometric plantar flexions, in that order, while the Maasai subjects began with the maximal isometric plantar flexion, before completing maximal jumps and repetitive jumps.

Maximal isometric plantar flexions were measured in a custom built chair. The chair was optimized for transport, but still rigid. As a control of ankle joint displacement, subjects tested in Norway had a wired electric goniometer (Noraxon Inc., Scottsdale, AZ, USA) attached to the top of their wrist and their lower leg. The ankle joint displacement was no larger than what you can expect from the displacement of mass on the foot during a maximal contraction. A wired load cell (Noraxon Inc., Scottsdale, AZ, USA) measured the force applied to the footplate. Subjects was asked to gradually increase the force for approximately three seconds before reaching maximal force. As maximal force was achieved they were told to decrease the force at the same rate of increase. All subjects needed test-trials in order to achieve maximal force. For Maasai subjects a near maximal force with a gradually inclining force was accepted as they were unfamiliar with the concept of maximal voluntary contractions. Data was captured in a program ran in MATLAB (R2015a, The MathWorks, Inc, Kista, Sweden).

During both the repetitive and maximal jumps, subjects were instructed to keep their hands on their hip at all times. Completing the maximal jumps (squat jumps and counter movement jumps) was no simple task for the Maasai subjects as it was far from movements they were familiar with. All subjects were allowed to practice the jumps. Counter movement jumps were done from a standing position to a preferred depth. During the squat jumps the subjects were all told by the same person to get to a position near 90 degrees of knee flexion. 2-3 successful trials were saved of both squat jumps and counter movement jumps with about a one-minute break in between jumps. For some Maasai subjects no squat jumps were recorded as it was not possible for some of them to get into the right position, and for some of them to complete the movement without a counter movement.

During the jumping trial subjects were first told to jump repeatedly at the pace they were most comfortable with. The subjects were asked if they were ready to jump, and if ready, the recording was not started until a controlled pace and a good rhythm was found. After one tracking the subjects got an approximated two minutes break as data was saved. After 2-3 successful submaximal trials subjects were told to jump maximally, but still repeatedly. During these jumps, keeping balance and staying inside of the force plate was more challenging, and subjects were therefore told to look at a point or an item to better control their landing. 2-3 successful maximal trials were captured.

Forespørsel om deltagelse i forsøksprosjekt

Dette er et spørsmål til deg om å delta i et forskningsstudie som omhandler hopp-prestasjon og muskel-sene-funksjon i underekstremiteten. Formålet er å sammenlikne Maasai-folket med nordiske atleter og kontroller. Prosjektet er et masterprosjekt ved Norges idrettshøgskole, og gjennomføres under veiledning av hovedveileder Jens Bojsen-Møller og biveileder Olivier Seynnes.

Bakgrunnen for studiet

Formålet med dette prosjektet er å sammenlikne hopp-prestasjon og muskel-sene funksjon i underekstremiteten til Maasai-folket med nordiske atleter og kontrollpersoner. Muskelaktivering, mekaniske egenskaper i sene og aponeurose, samt evnen til å lagre og frigjøre energi i kraftoverførende vev er alle viktige faktorer for hopp-prestasjon. Den direkte sammenhengen mellom disse faktorene er fremdeles uklart. Maasai-folket er et nomadefolk som lever i områder rundt grensen mellom Kenya og Tanzania. De praktiserer stammeritualer hvor det er observert ekstreme hopphøyder, >50cm. Det kan tenkes at deres livsstil, kultur og tradisjoner har bidratt til å oppnå ekstreme hopp-prestasjoner.

Hovedmål

(1) Undersøke hopp-prestasjon (maks hopphøyde og power i underekstremiteten) samt muskel-sene-funksjonen og biomekaniske egenskaper i de kraftbærende vevene i Maasai-folket.

(2) Sammenlikne disse dataene med tilsvarende data fra kontrollpersoner og erfarne atleter innen hoppidretter.

Hva inneholder studien?

Forsøkspersonene vil bli testet på laboratoriet for bevegelsesanalyse ved Norges idrettshøgskole. Testene som gjennomføres vil bestå av antropometriske målinger, senestivhet i akillessenen, samt maksimale og repetitive hopp.

Fordeler og ulemper

Deltakelsen i prosjektet krever noe tid og oppmerksomhet, da testingen tar i overkant av to timer. Vi vil tilpasse oppmøtet slik at det passer for forsøkspersonene. Forsøkspersonene må selv ta seg til Norges idrettshøgskole, og vil ikke få dekket reisekostnader. Det ønskes at forsøkspersonene ikke gjennomfører tung beintrening dagen før eller på selve testdagen, da dette kan påvirke testresultatene. Det er svært liten risiko for skade under gjennomføring av disse testene, men det kan forekomme stølhet de påfølgende dagene. Deltagelsen vil gi deg et innblikk i hvordan tester og forskning rundt fysiske egenskaper utføres. Du kan som forsøksperson til enhver tid, før og under studiet, trekke deg uten å oppgi begrunnelse.

Hva skjer med dine testresultater og opplysninger?

Alle personlige opplysninger, samt testresultatene, vil være avidentifisert under forsøksperioden og anonymisert når studien er gjennomført. Dette betyr at du ved forsøksstart vil få utdelt et forsøkspersonnummer som skal brukes under studien. Det er kun dette nummeret som vil være direkte knyttet til dine data. Listen som viser hvilket forsøkspersonnummer du har vil bli slettet når studien er ferdig. Da vil det ikke være mulig knytte dine data til din person. Det vil derfor ikke være mulig å identifisere deg i resultatene fra studien når disse senere publiseres.

Samtykke til deltagelse i studien

Om du har lest informasjonsskrivet og ønsker deltagelse i prosjektet, kan du signere "samtykke om deltagelse" nedenfor. Dersom du ønsker å trekke deg eller har noen spørsmål, ta kontakt med en av oss:

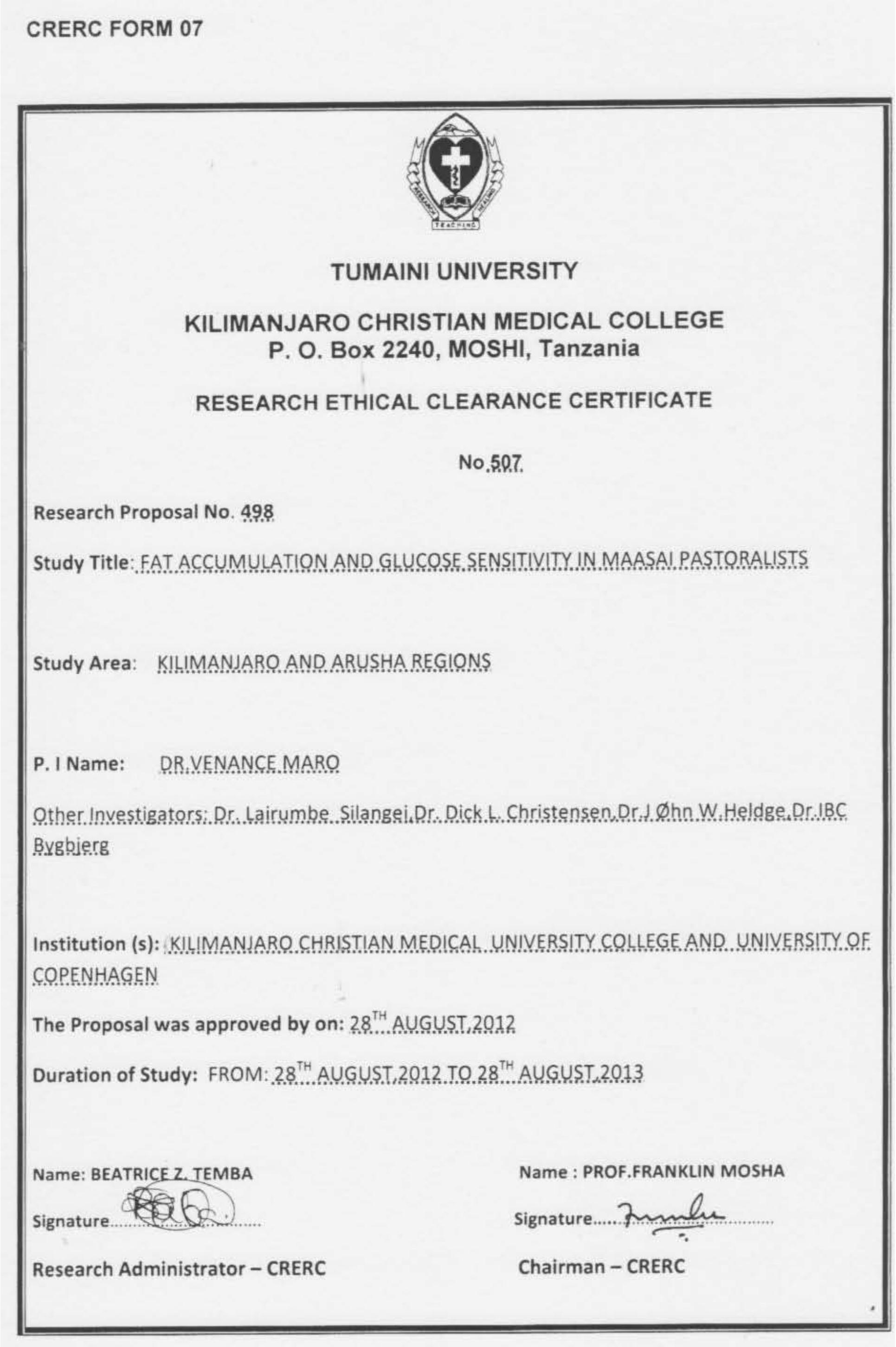
- Andreas Skiri Refsdal, 476 27 373	and-ref@online.no
- Herman Hernæs, 416 04 232	herman.hernaes@gmail.com
- Jens Bojsen-Møller, 23 26 22 34	jens.bojsen-moller@nih.no
- Olivier Seynnes, 23 26 23 08	olivier.seynnes@nih.no

Jeg er villig til å delta i studien

(signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(signert av testansvarlig, dato)



CRERC FORM 07

TUMAINI UNIVERSITY

KILIMANJARO CHRISTIAN MEDICAL COLLEGE
P. O. Box 2240, MOSHI, Tanzania

RESEARCH ETHICAL CLEARANCE CERTIFICATE

No.507

Research Proposal No. 498

Study Title: FAT ACCUMULATION AND GLUCOSE SENSITIVITY IN MAASAI PASTORALISTS

Study Area: KILIMANJARO AND ARUSHA REGIONS

P. I Name: DR.VENANCE.MARO

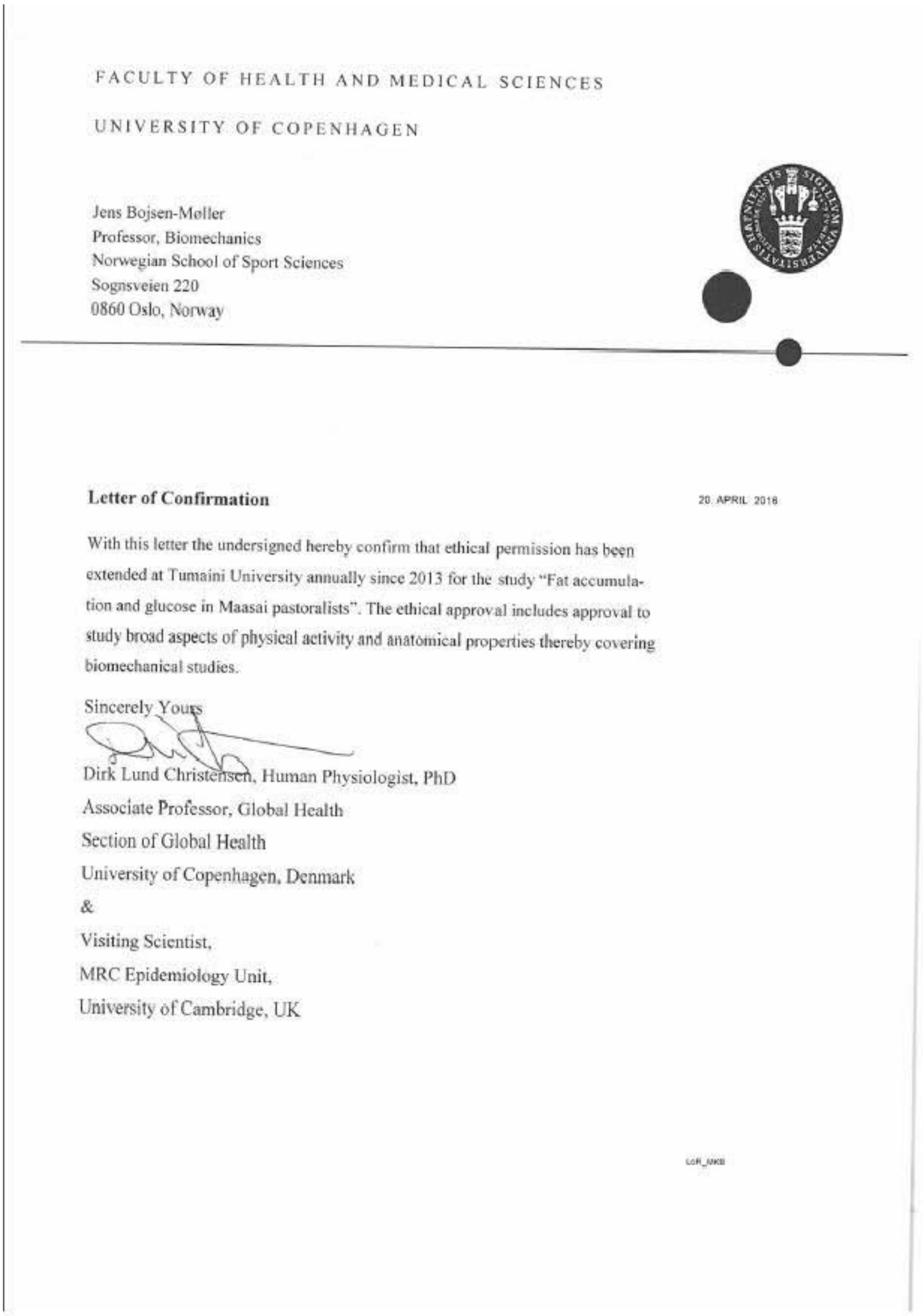
Other Investigators: Dr. Lairumbe Silangei,Dr. Dick L. Christensen,Dr.J Øhn W.Heldge,Dr.IBC Bygbjerg

Institution (s): KILIMANJARO CHRISTIAN MEDICAL UNIVERSITY COLLEGE AND UNIVERSITY OF COPENHAGEN

The Proposal was approved by on: 28TH AUGUST,2012

Duration of Study: FROM: 28TH AUGUST,2012 TO 28TH AUGUST,2013

Name: BEATRICE Z. TEMBA	**Name : PROF.FRANKLIN MOSHA**
Signature...............	**Signature**...............
Research Administrator – CRERC	**Chairman – CRERC**



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Professor, Biomechanics
Norwegian School of Sport Sciences
Sognsveien 220
0860 Oslo, Norway

Letter of Confirmation

20. APRIL 2016

With this letter the undersigned hereby confirm that ethical permission has been extended at Tumaini University annually since 2013 for the study "Fat accumulation and glucose in Maasai pastoralists". The ethical approval includes approval to study broad aspects of physical activity and anatomical properties thereby covering biomechanical studies.

Sincerely Yours

Dirk Lund Christensen, Human Physiologist, PhD
Associate Professor, Global Health
Section of Global Health
University of Copenhagen, Denmark
&
Visiting Scientist,
MRC Epidemiology Unit,
University of Cambridge, UK