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Title: Mechanical properties, physiological behavior and function of aponeurosis and tendon

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Abstract

During human movement, the muscle and tendinous structures interact as a mechanical system in which forces are generated and transmitted to the bone, and energy is stored and released to optimize function and economy of movement and/or to reduce risk of injury. The present review addresses certain aspects of how the anatomical design and mechanical and material properties of the force transmitting tissues contribute to the function of the muscle-tendon unit and thus overall human function. The force bearing tissues are examined from a structural macroscopic point of view down to the nanoscale level of the collagen fibril. In recent years, the understanding of *in vivo* mechanical function of the force bearing tissues has increased, and it has become clear that these tissues adapt to loading and unloading, and further that force transmission mechanics is more complex than previously thought. Future investigations of the force transmitting tissues in 3D will enable a greater understanding of the complex functional interplay between muscle and tendon with relevance for performance, injury mechanisms and rehabilitation strategies.

Introduction

The muscle-tendon unit can be seen as the basic functional entity of human movement capable of serving both as a motor, a damper and a spring to exert, dissipate or store and release energy respectively (1, 66). Such complex functions are made possible by serial and parallel coupling of active *force generating tissues* and passive *force transmitting tissues* and by taking advantage of the ability to shift energy between active and passive tissues during locomotion. The individual tissues of the muscle-tendon unit have been studied separately, but to comprehend human function in general, the functional interplay between these tissues must be understood. The present review discusses how the force transmitting tissues contribute to the function of the muscle-tendon unit and thus overall human function. The force bearing tissues are examined from a structural macroscopic point of view down to the nanoscale level of the collagen fibril.

1. Tendon structure

Anatomical design is complex and with large variation between separate muscle-tendon units (41). Generally, muscle fibers insert via extracellular matrix onto aponeuroses that in turn merge to become rope-like tendons of varying dimensions. The quadriceps and triceps surae, with the associated patella and Achilles tendon respectively, are important for human movement, and have been extensively researched in sports and human movement science. Nonetheless, these structures remain quite different in a functional perspective. While the Achilles tendon is long and relatively thin, the patella tendon is short and with a greater cross-sectional area (44, 45). The Achilles tendon has by far the greatest incidence of rupture (13, 47), illustrating that the Achilles tendon operates with high strain and closer to its breaking strength during daily function (40). The benefit of augmented strain however, is an

increased ability to store and release mechanical energy and to provide for energy dissipation when necessary. Moreover, tendon deformation or stiffness has been shown to modulate contractile force production or efficiency during dynamic efforts by influencing the operating length of the involved muscle fascicles (38, 49, 50). The Achilles tendon is organized in compartments that relate to the three corresponding muscles of the muscle-tendon unit (24, 85), which suggests that the Achilles tendon may be loaded heterogeneously during locomotion, and further that deformation may differ between tendon compartments (7, 8, 12, 15, 16, 18, 30, 78) (Fig. 1). Conversely, the patella tendon origins partly from and inserts into bone, and the tendon fascicles are organized more in parallel compared to the Achilles tendon. Although intra-tendinous differences in patella tendon material properties have been observed (36), and studies have observed non-uniform patella tendon strain during (*ex vivo*) loading (4, 10) these studies show conflicting results, and it appears likely that the patella tendon undergoes more homogenous loading and deformation *in vivo* compared to the Achilles tendon. A special feature of the Achilles tendon is its internal rotation from proximal to distal which perhaps facilitates more optimal strain and energy storage during loading (17, 21, 24, 85) (Fig. 2). Both tendon compartmentalization and longitudinal tendon rotation is subject to large inter-individual variability (24), which in turn may reflect individual performance and/or injury risk. Tendon design seemingly reflects function and it is likely that a trade-off exists between the ability to store and release energy on one side, and force transmission, contractile efficiency and position control on the other (40). It should be noted that the free tendon remains just one single part of the force transmitting tissues, and that also the more proximal aponeurosis may play a role for muscle-tendon unit function.

2. Aponeurosis

During locomotion, it is known that tendinous tissues are stretched and energy is stored, and subsequently converted into kinetic energy when unloaded (2, 14, 67), however, to what extent human aponeurosis and free tendon behave in a similar fashion and therefore contribute to energy storage in the same way remains largely unanswered.

In contrast to the free tendon, the mechanical properties of the aponeurosis have not received a great deal of attention in the literature. Some early studies examined strain properties along the length of muscle-tendon unit in animal models (frog, semitendinosus), and these data show that during passive loading the tensile strain of the aponeurosis exceeded that of the free tendon (51, 52), or that it was similar in the gastrocnemius muscle-tendon unit (89). It should be noted that the actual thickness of the aponeurosis changes along its length such that it becomes thicker closer to the free tendon (70), and therefore the strain may also differ, which has been shown in both human soleus and frog gastrocnemius muscle (27, 89). Because the muscle inserts onto the aponeurosis, it is conceivable that the force of the muscle contraction may influence the aponeurosis behavior along its length. It has been shown that the free tendon and aponeurosis in cat soleus muscle have equivalent mechanical properties during contraction (70), or that the stiffness of the aponeurosis is less than that of the free tendon (23). However, when comparing the aponeurosis strain in frog semitendinosus muscle during passive loading and that during muscle contraction, the aponeurosis strain was far less (14%) relative to strain during passive loading (24%), which indicates that the muscle contraction *per se* changes the aponeurosis strain behavior (52).

Human data on aponeurosis mechanical behavior *in vivo* have been relatively scarce, but recent developments in the imaging field have permitted the question to be investigated. Using ultrasonography it has been shown that the strain of the aponeurosis is greater than that of the tendon of the low stress anterior tibialis muscle-tendon unit in the longitudinal direction during electrical stimulation to sub-maximal forces (56). In contrast, it has been shown that the strain of the aponeurosis and tendon of the human gastrocnemius muscle are similar (60), although in this study the aponeurosis of the soleus muscle was unaccounted for. It is possible that these differences relate to muscle architecture and functional demands. Using the ultrasonography method coupled with a needle inserted into the free Achilles tendon as a marker, it has been shown that longitudinal strain for the tendon (8%) far exceeded that of the aponeurosis (1.4%)(57), which would suggest that the tendon and aponeurosis have different functional roles during *in vivo* force transmission.

Importantly, the human aponeurosis architecture can be rather intricate, which is not readily captured with 2D ultrasonography, but is better suited for magnetic resonance imaging. With respect to the soleus muscle, for example, it has been shown that architecture is quite complex and that tissue velocity mapping during contraction varies throughout the aponeurosis (26). Moreover, using cine phase-contrast magnetic resonance images it has been shown that the Achilles tendon strains 4.7% at 40% of maximal voluntary contraction, while the mid-region of the aponeurosis, below the gastrocnemius insertion, strained 2.2%, and the distal aponeurosis shortened 2.5% (28), which supports the notion that that during contraction the aponeurosis strains less in the longitudinal direction than the free tendon (52, 57).

It has been suggested that longitudinal aponeurosis deformation is less and lateral deformation is greater during contractile loading compared to that during passive tensile loading (96). The vast majority of studies have examined the strain behavior in the longitudinal direction, but this overlooks the possibility that the aponeurosis can be loaded in the transverse direction. Using X-ray imaging technique and radio-opaque markers that tracked aponeurosis shape of the gastrocnemius in turkeys it was possible to demonstrate that muscle contraction caused the aponeurosis to expand both in the longitudinal and transverse direction relative to the muscle line of action (6). In fact, transverse strains were on average 4 times greater than longitudinal strains (9). Therefore, it seems that the muscle fibers that connect and anchor to the aponeurosis can exert both transverse forces due to expansion of shortening muscle fibers as well as longitudinal forces (6, 9), which can serve to augment aponeurosis stiffness in the longitudinal direction (Fig. 3). Increased understanding of the functional consequences of multidimensional aponeurosis strain during loading is required in future work.

3. Intra-tendinous force transmission

Tendon fascicles. Imaging and histopathology findings indicate that tendon injury mostly concerns a particular region of the tendon, e.g. the posterior portion of the patellar tendon (42), and this has prompted the question if the mechanical properties of this particular region differs from that of other regions of the tendon (35). Cadaveric studies examining strain during loading of whole patella tendon have yielded conflicting results with greater strain anteriorly (4) or greater strain posteriorly (10) of the patellar tendon. An alternative approach to examine regional differences has been to mechanically test isolated fascicles

(35): These data suggest that the anterior portion of the human patellar tendon in young men displayed far greater peak and yield stress and tangent modulus compared to the posterior portion of the tendon, indicating region specific material properties (35). This study was subsequently repeated and the findings were confirmed, and moreover showed that there were differences in collagen crosslink composition and fibril diameter, although these differences appeared to be unrelated to the mechanical properties (34).

The fact that the tendon fascicle is a distinct entity within the tendon (39) and that fascicles appear to differ in composition and mechanical behavior (34, 35, 88) have prompted investigations in to what extent tendon fascicle are independent force transmitting structures, or whether forces can be transmitted in parallel between adjacent tendon fascicles similar to parallel force transmission known to occur between muscle fibers. This was originally examined in cutting experiments of adjoining collagen fascicles obtained from the anterior human patellar tendon and Achilles tendon (37). Severing one of the two connected fascicles markedly reduced the stiffness, and when both fascicles were cut such that only lateral force transmission was possible via interfascicular connective tissue during specimen deformation, very little force was registered, indicating minor lateral force transmission between adjacent tendon fascicles (Fig. 4.A). This would also indirectly suggest that the human fascicle is an independent force transmitting structure that spans the length of the tendon (11, 25, 37). This apparent independence on part of the fascicles may be necessary to accommodate a changing force–length relationship of the fascicle (patellar tendon) with altered joint angle and/or heterogeneous muscle activation (Achilles tendon) (37). The differential human intratendinous movement, *in vivo*, has been demonstrated using dedicated ultrasound techniques (7, 30, 65). Interestingly, it should be noted that

more recently a similar approach of testing interfascicular force transmission has been used in animal models (horse, rat tail and porcine), and those data indicate that there is considerable more force transfer between adjacent fascicles (43, 88) than previously reported in human tendon (37). It also appears that horse fascicles do not to continue as separate entities within the tendon (3). It is difficult to reconcile these dissimilarities, but they may relate to species and methodological differences. In this context, it is also worth noting that tendon fascicles within a species may behave mechanically different. For example, fascicles from the patellar tendon appear to be far stiffer than that of the Achilles at a given strain (37), and in horse it has been shown that energy storing superficial digital flexor tendon is less stiff than the positional common digital extensor tendon (88). Albeit speculative, it appears that tendon properties are related perhaps to the function of the specific muscle-tendon unit (force transmission, energy storage) or perhaps related also to mechanical constaints of function where the direction of tendon load in some tendons change through the joint ROM.

Tendon fibril. At collagen fibril level the specific pathway of force transmission remains largely unresolved. The collagen fibrils of tendon, which have a diameter ranging from 30 to 200 nm (48, 58, 94), are considered the principle load transmitting structure of tendons. The extra-cellular matrix surrounds the fibrils and consists of water, proteoglycans, glycosaminoglycans (GAGs), elastin and glycoproteins (91). Uneven fiber deformation at the microscopic level (72-75), and x-ray diffraction studies demonstrating that fibrils are deformed less than the whole structure (31, 46, 63) support the notion that extrafibrillar components transfer force between neighboring fibrils. It has been suggested that lateral force transmission occurs via proteoglycans and their associated GAG chains, including

chondroitin- and dermatan-sulfate (68, 69). However, removing this complex in human tendon (80) and ligament (54, 55) does not appreciably affect the mechanical properties of the tissue and therefore indicates that they do not contribute in a meaningful way to lateral force transmission. Yet, it has been shown that some sliding can occur at the fiber level, which can result in strain at the cellular level (<2%) with implications for mechanotransduction and viscoelastic behavior (71, 72).

The notion that there is a lateral pathway of force transmission largely rests on the assumption that fibrils in mature tissue are discontinuous but determining the actual structural length of fibrils with a diameter of 30-200 nm has proven rather challenging. Studies based on direct structural studies suggest that fibrils may be structurally continuous while those based on indirect methods tend to support that fibrils are discontinuous (20, 29, 62, 77, 86). In a recent study serial block face-scanning electron microscopy was used to investigate tissue from adult human patellar and hamstring tendons, and it was possible to track 2700 fibrils over a defined distance and only detect one single fibril tip (82) (Fig. 4). In fact, reports of collagen fibril ends within mature human tendon are extremely rare. Based on these data it was estimated that the fibril length was 67.5 mm. In the same study, it was possible to track the full length of continuous fibrils from muscle to bone in a short animal tendon (mouse stapedius,125 μm), and collectively these data suggest that fibrils are likely continuous (82).

The mechanical behavior of tendon has for decades been assessed using whole tendon using animal or cadaver tissue *ex vivo*, or more recently using ultrasonography-based methodology, *in vivo* (32, 57). Mechanical behavior of the individual fibril has only been

possible in recent years (53, 64, 79, 81, 83, 95). In one of the first studies, atomic force microscopy was used to test single fibrils and it was shown that similar to that of whole tendon individual fibrils from human patellar tendon display typical viscoelastic behavior when tested at different strain rates (81). The stress-strain behavior of whole tendon typically includes an initial toe-region with a low modulus that transitions to a linear region with much higher modulus before reaching the yield and fracture point. However, it appears that single human fibrils tested to failure behave differently (83): they display a unique three-phase stress-strain behavior with an initial rise in modulus followed by a plateau with reduced modulus, which is finally followed by an even greater increase in stress and modulus before reaching the fracture point (64, 83, 84). The mechanism for such a three-phase behavior is unknown, but may include helix uncoiling and molecular slippage (90), and may serve to increase toughness of the fibril and reducing the risk of brittle failure.

4. Physiological behavior of tendons

It is clear from the above that the distinct mechanical or physiological function of the force transmitting tissues during human movement remains complex both at the macroscopic and microscopic level. Below are considerations with respect to further research in the area.

It is clear that on a species and individual level the tendon and muscle dimensions and properties develop in a close interplay most likely governed by the ability of the muscle to exert force (40, 41). Optimal material properties of the force bearing tissues is thus regulated by daily loading (93) and this notion has relevance for performance but also for the understanding of injury mechanisms/etiology. For example, recent studies suggest that

the mechanical properties of the force bearing tissues play a role for 'explosive' type MTU contraction as tendon stiffness seems to be related to rate of force development in rapid muscle action (19, 59), and concomitantly a number of studies have demonstrated increased tendon stiffness following strength training (5, 45, 76, 92). It follows that the force bearing tissues are more plastic and undergo adaptation following habitual loading (e.g. induced by strength training) than was previously thought.

While the basis for tendon compartmentalization may lie in embryologic development, the functional relevance hereof, and/or the possibility for non-uniform tendon deformation is not well known. However, in the case of the triceps surae and Achilles tendon, it can be speculated that compartmentalization contributes to improved or optimized function during complex locomotion tasks by enabling a more precise exertion of joint moments from the different muscle actuators that span the ankle and subtalar joints (18).
Compartmentalization with respect to tissue mechanical properties or non-uniform loading of the patella tendon remains to be investigated. Perhaps compartmentalization is irrelevant for the patella tendon since it originates from and inserts onto bone. However, the anterior portion of the patella tendon where tendon fascicles cross the patella can be seen as a continuation of the more proximal quadriceps tendon. In line with this notion there are data demonstrating anterio-posterior differences in patellar tendon material properties (33, 36, 79), which suggests that perhaps there is tendon compartmentalization and non-uniform loading. Nonetheless, whether compartmentalization in the patella tendon has functional consequences, for example in relation to stress concentrations and or injury mechanisms, remains to be examined.

The functional relevance of tendon rotation remains unknown but different explanations can be suggested: 1) Tendon rotation may enable muscle fibers situated far apart to operate at more similar and/or more optimal lengths with respect to force exertion during the full joint range of motion. One previous modelling study examined masseter muscles in fish where muscle and tendon rotation occur. It was shown that in broad muscles that operate at distance from the joint axis of rotation, and where the tendon enthesis covers a wide area, variation in the muscle fibers ability to exert force occurred due to inter-individual differences in relative fiber length through the joint range of motion. A rotated tendon, or a design where the muscle fibers rotate or cross each other, was shown to equalize force-length properties for muscle fibers, thereby optimizing contractile ability (22). The human triceps surae is in fact a broad muscle that operates at distance from the joint compared to other ankle joint muscles, and the Achilles tendon does have a broad insertional area on the calcaneus perhaps suggesting a similar mechanism for this muscle. 2) Rotation may facilitate the ability to store and release energy during loading and unloading in a similar manner to that of rope design since one feature of twisted ropes is to enhance the ability to strain and store energy (16, 61). A rope analogy may seem reasonable for human tendon design since some tendons are more involved in position control and effective force transmission, such as for example tendons of the hand or forearm, while the Achilles tendon is highly involved in energy storage and release during locomotion. One study reported greater maximal strain and strength but surprisingly increased stiffness in a cadaver model where patella tendons were twisted 90°, which underscores that additional studies are required to elucidate the relevance of tendon rotation (87). 3) Finally, tendon rotation could serve to regulate intratendinous pressure during the high stresses and strains. In a coherent or linear structure, internal pressure is reduced more under tensile stress compared to a rotated

structure. Albeit highly speculative, modulation of intratendinous pressure may play a role for maintaining vessel and nerve function, and potential fluid diffusion that in turn may relate to the health and function of the tissue.

Conclusion / perspectives

In conclusion, developments in both imaging technology and application thereof in *in vivo* studies, but also studies at nanoscale have increased the understanding of the function of the force bearing tissues. It has become clear that these tissues respond to habitual loading, and moreover that force transmission is more complex than previously assumed. Notably, at collagen fibril level, it seems that failure and material properties appear different from that observed at the whole tendon level. At tendon fascicle level, it seems that force may be directed non-uniformly into the tendon, and that at least some tendons undergo heterogeneous loading with associated inter fascicle sliding as a result. At tendon structure level, it seems at least in some tendons, that anatomical design such as tendon compartmentalization and even tendon rotation may contribute to the mechanical function or performance of the tendon structure in the interplay with contractile tissues. Many studies have examined the force bearing tissues in two dimensions, however, recent advances in imaging has enabled three dimensional investigations and it seems clear that the next steps in future research must be to achieve a detailed understanding of function during loading of the force bearing tissues, and the muscle-tendon unit in 3D. Such understanding will have great relevance for the understanding of injury mechanisms, for developing strategies for rehabilitation from injury and injury prevention, and not the least for performance enhancement and development in sports and exercise training.

Figure legends

Figure 1: Non-uniform Achilles tendon displacement during loading as examined with ultrasonography (speckle tracking) and verified in the same study by tracking of tantalum beads surgically placed into the tendon. Panel A: Sagittal image of the Achilles tendon where 4 regions of interest (layers) were identified. Panel B: Non-uniform displacement of intratendinous tissues where superficial (posterior) layers underwent appr. 20% less displacement compared to deeper layers of the tendon. Fig modified from Beyer et al 2018 (12)

Figure 2: Achilles tendon rotation. Panel A: Tendon rotation as noted in 1894 by F. G. Parsons in different mammals (including humans)(61). Panel B: Illustration of the rotation of the human Achilles tendon. Arrows show that tendon fascicle bundles that originate from the medial gastrocnemius (GM) insert on the postero-lateral side of the calcaneus, while fascicle bundles from the lateral gastrocnemius (GL) insert on the lateral-anterior aspect of the insertion site. Oval cartoons depict cross sections of the tendon at a proximal site (upper drawing) and just above the calcaneal insertion (lower drawing). From Bojsen-Moller and Magnusson 2015 (18)

Figure 3: During active muscle contraction (black lines), the aponeurosis lengthens and widens (B). During passive loading (grey lines), both muscle fibers and aponeurosis lengthens while the aponeurosis width decreases (B). The study illustrates that the aponeurosis follows

a different force-length profile during active loading versus passive stretch. From (6) with permission.

Figure 4: Fascicle and fibril loading: A) Force–displacement data from a human patellar tendon specimen that was strained to ~3%. Cycle 1 represented two adjacent and intact fascicles with potential force transmission both longitudinally and laterally. In Cycle 2, one fascicle was cut (marked by small black notch) and thus the curve represents one intact fascicle that could potentially transmit force longitudinally and laterally, and one adjacent fascicle that could potentially transmit force laterally only via the intact fascicular membrane intact. In Cycle 3 both fascicles were cut at opposite ends (marked by small black notches), and thus the trace represents two adjacent fascicles that could potentially only transmit force laterally via the intact fascicular membrane. Adopted from (37). B) Stress-strain response during loading of a human patellar tendon collagen fibril. Three distinct regions can be observed that give rise to two distinct peak modulus values (from (83) with permission). C) Illustration of fibril tracing through the tendon. Serial block face-scanning electron microscopy 3D segment of a region with 300 individual collagen fibrils where no fibril ends are visualized (from (82) with permission).

References

1.   **Alexander RM**. Energy-saving mechanisms in walking and running. *J Exp Biol* 160: 55-69, 1991.
2.   **Alexander RM, and Bennet-Clark HC**. Storage of elastic strain energy in muscle and other tissues. *Nature* 265: 114-117, 1977.
3.   **Ali OJ, Comerford EJ, Clegg PD, and Canty-Laird EG**. Variations during ageing in the three-dimensional anatomical arrangement of fascicles within the equine superficial digital flexor tendon. *European cells & materials* 35: 87-102, 2018.
4.   **Almekinders LC, Vellema JH, and Weinhold PS**. Strain patterns in the patellar tendon and the implications for patellar tendinopathy. *Knee Surg Sports Traumatol Arthrosc* 10: 2-5, 2002.
5.   **Arampatzis A, Karamanidis K, and Albracht K**. Adaptational responses of the human Achilles tendon by modulation of the applied cyclic strain magnitude. *J Exp Biol* 210: 2743-2753, 2007.
6.   **Arellano CJ, Gidmark NJ, Konow N, Azizi E, and Roberts TJ**. Determinants of aponeurosis shape change during muscle contraction. *J Biomech* 49: 1812-1817, 2016.
7.   **Arndt A, Bengtsson AS, Peolsson M, Thorstensson A, and Movin T**. Non-uniform displacement within the Achilles tendon during passive ankle joint motion. *Knee Surg Sports Traumatol Arthrosc* 20: 1868-1874, 2012.
8.   **Arndt A, Bruggemann GP, Koebke J, and Segesser B**. Asymmetrical loading of the human triceps surae: II. Differences in calcaneal moments. *Foot & ankle international* 20: 450-455, 1999.
9.   **Azizi E, and Roberts TJ**. Biaxial strain and variable stiffness in aponeuroses. *The Journal of physiology* 587: 4309-4318, 2009.
10.   **Basso O, Amis AA, Race A, and Johnson DP**. Patellar tendon fiber strains: their differential responses to quadriceps tension. *Clin Orthop Relat Res* 246-253, 2002.
11.   **Basso O, Johnson DP, and Amis AA**. The anatomy of the patellar tendon. *Knee Surg Sports Traumatol Arthrosc* 9: 2-5, 2001.
12.   **Beyer R, Agergaard, A.-S., Magnusson, S. P., Svensson, R. B.** Speckle tracking in healthy and surgically repaired human
Achilles tendons at different knee angles—A validation using
implanted tantalum beads. *Transl Sports Med* 1: 79-88, 2018.
13.   **Beyer R, Kongsgaard M, Hougs Kjaer B, Ohlenschlaeger T, Kjaer M, and Magnusson SP**. Heavy Slow Resistance Versus Eccentric Training as Treatment for Achilles Tendinopathy: A Randomized Controlled Trial. *The American journal of sports medicine* 2015.
14.   **Biewener AA, and Roberts TJ**. Muscle and tendon contributions to force, work, and elastic energy savings: a comparative perspective. *Exerc Sport Sci Rev* 28: 99-107, 2000.
15.   **Bogaerts S, De Brito Carvalho C, Scheys L, Desloovere K, D'Hooge J, Maes F, Suetens P, and Peers K**. Evaluation of tissue displacement and regional strain in the Achilles tendon using quantitative high-frequency ultrasound. *PloS one* 12: e0181364, 2017.
16.   **Bojsen-Moller J, Hansen P, Aagaard P, Svantesson U, Kjaer M, and Magnusson SP**. Differential displacement of the human soleus and medial

gastrocnemius aponeuroses during isometric plantar flexor contractions in vivo. *Journal of applied physiology* 97: 1908-1914, 2004.

17.   **Bojsen-Moller J, Larsson B, and Aagaard P**. Physical requirements in Olympic sailing. *Eur J Sport Sci* 15: 220-227, 2015.

18.   **Bojsen-Moller J, and Magnusson SP**. Heterogeneous Loading of the Human Achilles Tendon In Vivo. *Exerc Sport Sci Rev* 43: 190-197, 2015.

19.   **Bojsen-Moller J, Magnusson SP, Rasmussen LR, Kjaer M, and Aagaard P**. Muscle performance during maximal isometric and dynamic contractions is influenced by the stiffness of the tendinous structures. *Journal of applied physiology* 99: 986-994, 2005.

20.   **Craig AS, Birtles MJ, Conway JF, and Parry DAD**. AN ESTIMATE OF THE MEAN LENGTH OF COLLAGEN FIBRILS IN RAT TAIL-TENDON AS A FUNCTION OF AGE. *Connect Tissue Res* 19: 51-62, 1989.

21.   **Cummins EJ, Anson BJ, and et al.** The structure of the calcaneal tendon (of Achilles) in relation to orthopedic surgery, with additional observations on the plantaris muscle. *Surgery, gynecology & obstetrics* 83: 107-116, 1946.

22.   **Dean MN, Azizi E, and Summers AP**. Uniform strain in broad muscles: active and passive effects of the twisted tendon of the spotted ratfish Hydrolagus colliei. *The Journal of experimental biology* 210: 3395-3406, 2007.

23.   **Delgado-Lezama R, Raya JG, and Munoz-Martinez EJ**. Methods to find aponeurosis and tendon stiffness and the onset of muscle contraction. *J Neurosci Methods* 78: 125-132, 1997.

24.   **Edama M, Kubo M, Onishi H, Takabayashi T, Inai T, Yokoyama E, Hiroshi W, Satoshi N, and Kageyama I**. The twisted structure of the human Achilles tendon. *Scandinavian journal of medicine & science in sports* 2014.

25.   **Fallon J, Blevins FT, Vogel K, and Trotter J**. Functional morphology of the supraspinatus tendon. *J Orthop Res* 20: 920-926, 2002.

26.   **Finni T, Hodgson JA, Lai AM, Edgerton VR, and Sinha S**. Mapping of movement in the isometrically contracting human soleus muscle reveals details of its structural and functional complexity. *J Appl Physiol* 95: 2128-2133, 2003.

27.   **Finni T, Hodgson JA, Lai AM, Edgerton VR, and Sinha S**. Nonuniform strain of human soleus aponeurosis-tendon complex during submaximal voluntary contractions in vivo. *Journal of applied physiology* 95: 829-837, 2003.

28.   **Finni T, Hodgson JA, Lai AM, Edgerton VR, and Sinha S**. Nonuniform strain of human soleus aponeurosis-tendon complex during submaximal voluntary contractions in vivo. *J Appl Physiol* 95: 829-837, 2003.

29.   **Folkhard W, Geercken W, Knorzer E, Mosler E, Nemetschekgansler H, Nemetschek T, and Koch MHJ**. Structural Dynamic of Native Tendon Collagen. *J Mol Biol* 193: 405-407, 1987.

30.   **Franz JR, Slane LC, Rasske K, and Thelen DG**. Non-uniform in vivo deformations of the human Achilles tendon during walking. *Gait Posture* 41: 192-197, 2015.

31.   **Fratzl P, Misof K, Zizak I, Rapp G, Amenitsch H, and Bernstorff S**. Fibrillar structure and mechanical properties of collagen. *J Struct Biol* 122: 119-122, 1998.

32.   **Fukashiro S, Itoh M, Ichinose Y, Kawakami Y, and Fukunaga T**. Ultrasonography gives directly but noninvasively elastic characteristic of human tendon in vivo. *Eur J Appl Physiol Occup Physiol* 71: 555-557, 1995.

33.   **Hansen P, Haraldsson BT, Aagaard P, Kovanen V, Avery NC, Qvortrup K, Larsen JO, Krogsgaard M, Kjaer M, and Peter Magnusson S**. Lower strength of the

human posterior patellar tendon seems unrelated to mature collagen cross-linking and fibril morphology. *Journal of applied physiology* 108: 47-52, 2010.

34.   **Hansen P, Haraldsson BT, Aagaard P, Kovanen V, Avery NC, Qvortrup K, Larsen JO, Krogsgaard M, Kjaer M, and Peter Magnusson S**. Lower strength of the human posterior patellar tendon seems unrelated to mature collagen cross-linking and fibril morphology. *J Appl Physiol* 108: 47-52, 2010.

35.   **Haraldsson BT, Aagaard P, Krogsgaard M, Alkjaer T, Kjaer M, and Magnusson SP**. Region-specific mechanical properties of the human patella tendon. *J Appl Physiol* 98: 1006-1012, 2005.

36.   **Haraldsson BT, Aagaard P, Krogsgaard M, Alkjaer T, Kjaer M, and Magnusson SP**. Region-specific mechanical properties of the human patella tendon. *Journal of applied physiology* 98: 1006-1012, 2005.

37.   **Haraldsson BT, Aagaard P, Qvortrup K, Bojsen-Moller J, Krogsgaard M, Koskinen S, Kjaer M, and Magnusson SP**. Lateral force transmission between human tendon fascicles. *Matrix Biol* 27: 86-95, 2008.

38.   **Hirayama K, Iwanuma S, Ikeda N, Yoshikawa A, Ema R, and Kawakami Y**. Plyometric Training Favors Optimizing Muscle-Tendon Behavior during Depth Jumping. *Front Physiol* 8: 16, 2017.

39.   **Kastelic J, Galeski A, and Baer E**. The multicomposite structure of tendon. *Connect Tissue Res* 6: 11-23, 1978.

40.   **Ker RF**. The design of soft collagenous load-bearing tissues. *J Exp Biol* 202: 3315-3324, 1999.

41.   **ker RF, Alexander, R. McN, Bennet, M. B.** Why are mammalian tendons so thick? *J Zool* 216: 309-324, 1988.

42.   **Khan KM, Bonar F, Desmond PM, Cook JL, Young DA, Visentini PJ, Fehrmann MW, Kiss ZS, O'Brien PA, Harcourt PR, Dowling RJ, O'Sullivan RM, Crichton KJ, Tress BM, and Wark JD**. Patellar tendinosis (jumper's knee): findings at histopathologic examination, US, and MR imaging. Victorian Institute of Sport Tendon Study Group. *Radiology* 200: 821-827, 1996.

43.   **Kondratko-Mittnacht J, Duenwald-Kuehl S, Lakes R, and Vanderby R, Jr.** Shear load transfer in high and low stress tendons. *J Mech Behav Biomed Mater* 45: 109-120, 2015.

44.   **Kongsgaard M, Aagaard P, Kjaer M, and Magnusson SP**. Structural Achilles tendon properties in athletes subjected to different exercise modes and in Achilles tendon rupture patients. *J Appl Physiol* 99: 1965-1971, 2005.

45.   **Kongsgaard M, Reitelseder S, Pedersen TG, Holm L, Aagaard P, Kjaer M, and Magnusson SP**. Region specific patellar tendon hypertrophy in humans following resistance training. *Acta Physiol (Oxf)* 191: 111-121, 2007.

46.   **Krauss S, Fratzl P, Seto J, Currey JD, Estevez JA, Funari SS, and Gupta HS**. Inhomogeneous fibril stretching in antler starts after macroscopic yielding: indication for a nanoscale toughening mechanism. *Bone* 44: 1105-1110, 2009.

47.   **Kujala UM, Sarna S, and Kaprio J**. Cumulative incidence of achilles tendon rupture and tendinopathy in male former elite athletes. *Clin J Sport Med* 15: 133-135, 2005.

48.   **Lavagnino M, Arnoczky SP, Frank K, and Tian T**. Collagen fibril diameter distribution does not reflect changes in the mechanical properties of in vitro stress-deprived tendons. *J Biomech* 38: 69-75, 2005.

49.   **Lichtwark GA, and Wilson AM**. Is Achilles tendon compliance optimised for maximum muscle efficiency during locomotion? *J Biomech* 40: 1768-1775, 2007.

50.   **Lichtwark GA, and Wilson AM**. Optimal muscle fascicle length and tendon stiffness for maximising gastrocnemius efficiency during human walking and running. *J Theor Biol* 252: 662-673, 2008.

51.   **Lieber RL, Leonard ME, Brown CG, and Trestik CL**. Frog semitendinosis tendon load-strain and stress-strain properties during passive loading. *Am J Physiol* 261: C86-92, 1991.

52.   **Lieber RL, Leonard ME, and Brown-Maupin CG**. Effects of muscle contraction on the load-strain properties of frog aponeurosis and tendon. *Cells Tissues Organs* 166: 48-54, 2000.

53.   **Liu Y, Ballarini R, and Eppell SJ**. Tension tests on mammalian collagen fibrils. *Interface Focus* 6: 20150080, 2016.

54.   **Lujan TJ, Underwood CJ, Henninger HB, Thompson BM, and Weiss JA**. Effect of dermatan sulfate glycosaminoglycans on the quasi-static material properties of the human medial collateral ligament. *J Orthop Res* 25: 894-903, 2007.

55.   **Lujan TJ, Underwood CJ, Jacobs NT, and Weiss JA**. Contribution of glycosaminoglycans to viscoelastic tensile behavior of human ligament. *J Appl Physiol* 106: 423-431, 2009.

56.   **Maganaris CN, and Paul JP**. Load-elongation characteristics of in vivo human tendon and aponeurosis. *J Exp Biol* 203: 751-756, 2000.

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

58.   **Magnusson SP, Qvortrup K, Larsen JO, Rosager S, Hanson P, Aagaard P, Krogsgaard M, and Kjaer M**. Collagen fibril size and crimp morphology in ruptured and intact Achilles tendons. *Matrix Biol* 21: 369-377, 2002.

59.   **Mayfield DL, Cresswell AG, and Lichtwark GA**. Effects of series elastic compliance on muscle force summation and the rate of force rise. *The Journal of experimental biology* 219: 3261-3270, 2016.

60.   **Muramatsu T, Muraoka T, Takeshita D, Kawakami Y, Hirano Y, and Fukunaga T**. Mechanical properties of tendon and aponeurosis of human gastrocnemius muscle in vivo. *J Appl Physiol* 90: 1671-1678, 2001.

61.   **Parsons FG**. On the Morphology of the Tendo-Achillis. *Journal of anatomy and physiology* 28: 414-418, 1894.

62.   **Provenzano PP, and Vanderby R**. Collagen fibril morphology and organization: Implications for force transmission in ligament and tendon. *Matrix Biol* 25: 71-84, 2006.

63.   **Puxkandl R, Zizak I, Paris O, Keckes J, Tesch W, Bernstorff S, Purslow P, and Fratzl P**. Viscoelastic properties of collagen: synchrotron radiation investigations and structural model. *Philos Trans R Soc Lond B Biol Sci* 357: 191-197, 2002.

64.   **Quigley AS, Bancelin S, Deska-Gauthier D, Legare F, Kreplak L, and Veres SP**. In tendons, differing physiological requirements lead to functionally distinct nanostructures. *Scientific reports* 8: 4409, 2018.

65.   **R. B, A.-S. A, P. MS, and B. SR**. Speckle tracking in healthy and surgically repaired human Achilles tendons at different knee angles—A validation using implanted tantalum beads. *Translational Sports Medicine* 1: 79-88, 2018.

66.   **Roberts TJ, and Azizi E**. Flexible mechanisms: the diverse roles of biological springs in vertebrate movement. *The Journal of experimental biology* 214: 353-361, 2011.

67.   **Roberts TJ, Marsh RL, Weyand PG, and Taylor CR**. Muscular force in running turkeys: the economy of minimizing work. *Science* 275: 1113-1115, 1997.

68.   **Ryan CN, Sorushanova A, Lomas AJ, Mullen AM, Pandit A, and Zeugolis DI**. Glycosaminoglycans in Tendon Physiology, Pathophysiology, and Therapy. *Bioconjug Chem* 26: 1237-1251, 2015.

69.   **Scott JE, and Thomlinson AM**. The structure of interfibrillar proteoglycan bridges (shape modules') in extracellular matrix of fibrous connective tissues and their stability in various chemical environments. *J Anat* 192 (Pt 3): 391-405, 1998.

70.   **Scott SH, and Loeb GE**. Mechanical properties of aponeurosis and tendon of the cat soleus muscle during whole-muscle isometric contractions. *J Morphol* 224: 73-86, 1995.

71.   **Screen HR**. Investigating load relaxation mechanics in tendon. *J Mech Behav Biomed Mater* 1: 51-58, 2008.

72.   **Screen HR, Lee DA, Bader DL, and Shelton JC**. An investigation into the effects of the hierarchical structure of tendon fascicles on micromechanical properties. *Proc Inst Mech Eng H* 218: 109-119, 2004.

73.   **Screen HRC**. Investigating load relaxation mechanics in tendon. *J Mech Behav Biomed Mater* 1: 51-58, 2008.

74.   **Screen HRC, Bader DL, Lee DA, and Shelton JC**. Local strain measurement within tendon. *Strain* 40: 157-163, 2004.

75.   **Screen HRC, Lee DA, Bader DL, and Shelton JC**. An investigation into the effects of the hierarchical structure of tendon fascicles on micromechanical properties. *Proc Inst Mech Eng Part H-J Eng Med* 218: 109-119, 2004.

76.   **Seynnes OR, Erskine RM, Maganaris CN, Longo S, Simoneau EM, Grosset JF, and Narici MV**. Training-induced changes in structural and mechanical properties of the patellar tendon are related to muscle hypertrophy but not to strength gains. *Journal of applied physiology* 107: 523-530, 2009.

77.   **Silver FH, Christiansen DL, Snowhill PB, and Chen Y**. Role of storage on changes in the mechanical properties of tendon and self-assembled collagen fibers. *Connect Tissue Res* 41: 155-164, 2000.

78.   **Slane LC, and Thelen DG**. Non-uniform displacements within the Achilles tendon observed during passive and eccentric loading. *J Biomech* 47: 2831-2835, 2014.

79.   **Svensson RB, Hansen P, Hassenkam T, Haraldsson BT, Aagaard P, Kovanen V, Krogsgaard M, Kjaer M, and Magnusson SP**. Mechanical properties of human patellar tendon at the hierarchical levels of tendon and fibril. *J Appl Physiol* 112: 419-426, 2012.

80.   **Svensson RB, Hassenkam T, Hansen P, Kjaer M, and Magnusson SP**. Tensile force transmission in human patellar tendon fascicles is not mediated by glycosaminoglycans. *Connect Tissue Res* 52: 415-421, 2011.

81.   **Svensson RB, Hassenkam T, Hansen P, and Magnusson SP**. Viscoelastic behavior of discrete human collagen fibrils. *Journal of the Mechanical Behavior of Biomedical Materials* 3: 112-115, 2010.

82.   **Svensson RB, Herchenhan A, Starborg T, Larsen M, Kadler KE, Qvortrup K, and Magnusson SP**. Evidence of structurally continuous collagen fibrils in tendons. *Acta Biomater* 50: 293-301, 2017.

83.   **Svensson RB, Mulder H, Kovanen V, and Magnusson SP**. Fracture mechanics of collagen fibrils: influence of natural cross-links. *Biophys J* 104: 2476-2484, 2013.

84.   **Svensson RB, Smith ST, Moyer PJ, and Magnusson SP**. Effects of maturation and advanced glycation on tensile mechanics of collagen fibrils from rat tail and Achilles tendons. *Acta Biomater* 2018.

85.   **Szaro P, Witkowski G, Smigielski R, Krajewski P, and Ciszek B**. Fascicles of the adult human Achilles tendon - an anatomical study. *Ann Anat* 191: 586-593, 2009.

86.   **Szczesny SE, Caplan JL, Pedersen P, and Elliott DM**. Quantification of Interfibrillar Shear Stress in Aligned Soft Collagenous Tissues via Notch Tension Testing. *Sci Rep* 5: 14649, 2015.

87.   **Thambyah A, Thiagarajan P, and Goh JC**. Biomechanical study on the effect of twisted human patellar tendon. *Clin Biomech (Bristol, Avon)* 15: 756-760, 2000.

88.   **Thorpe CT, Udeze CP, Birch HL, Clegg PD, and Screen HR**. Specialization of tendon mechanical properties results from interfascicular differences. *J R Soc Interface* 9: 3108-3117, 2012.

89.   **Trestik CL, and Lieber RL**. Relationship between Achilles tendon mechanical properties and gastrocnemius muscle function. *J Biomech Eng* 115: 225-230, 1993.

90.   **Uzel SG, and Buehler MJ**. Molecular structure, mechanical behavior and failure mechanism of the C-terminal cross-link domain in type I collagen. *J Mech Behav Biomed Mater* 4: 153-161, 2011.

91.   **Wang JHC**. Mechanobiology of tendon. *J Biomech* 39: 1563-1582, 2006.

92.   **Werkhausen A, Albracht K, Cronin NJ, Paulsen G, Bojsen-Moller J, and Seynnes OR**. Effect of Training-Induced Changes in Achilles Tendon Stiffness on Muscle-Tendon Behavior During Landing. *Front Physiol* 9: 794, 2018.

93.   **Wiesinger HP, Kosters A, Muller E, and Seynnes OR**. Effects of Increased Loading on In Vivo Tendon Properties: A Systematic Review. *Med Sci Sports Exerc* 47: 1885-1895, 2015.

94.   **Williams IF, Craig AS, Parry DAD, Goodship AE, Shah J, and Silver IA**. Development of collagen fibril organization and collagen crimp patterns during tendon healing. *Int J Biol Macromol* 7: 275-282, 1985.

95.   **Yang L, van der Werf KO, Dijkstra PJ, Feijen J, and Bennink ML**. Micromechanical analysis of native and cross-linked collagen type I fibrils supports the existence of microfibrils. *J Mech Behav Biomed Mater* 6: 148-158, 2012.

96.   **Zuurbier CJ, Everard AJ, van der Wees P, and Huijing PA**. Length-force characteristics of the aponeurosis in the passive and active muscle condition and in the isolated condition. *J Biomech* 27: 445-453, 1994.
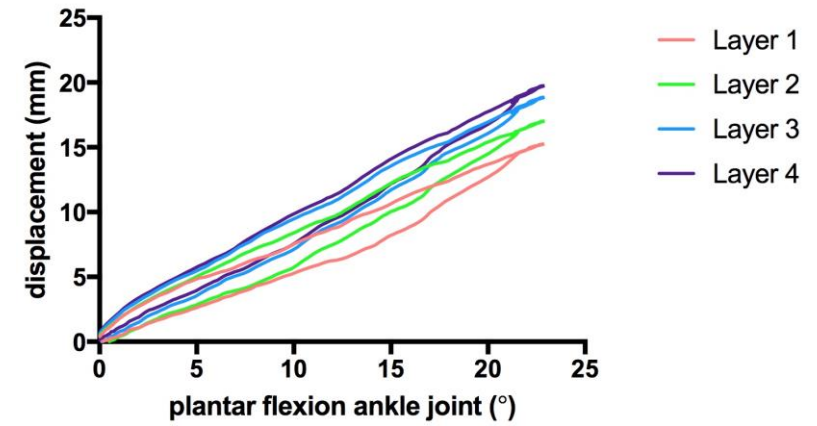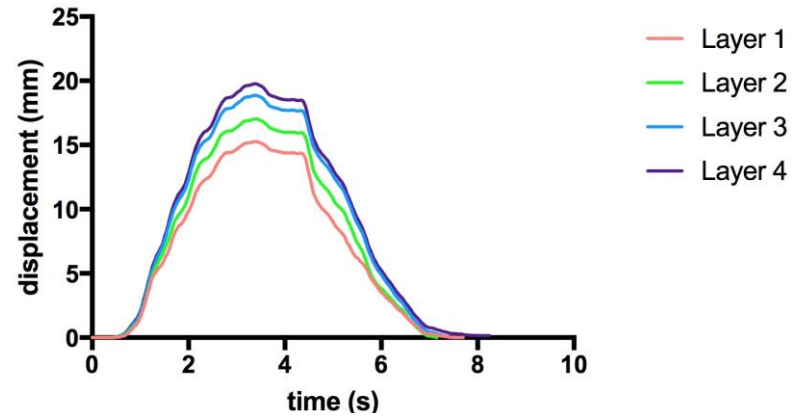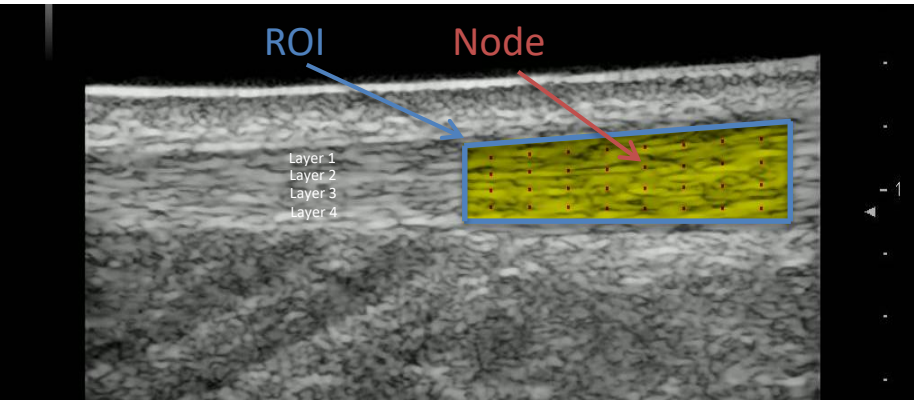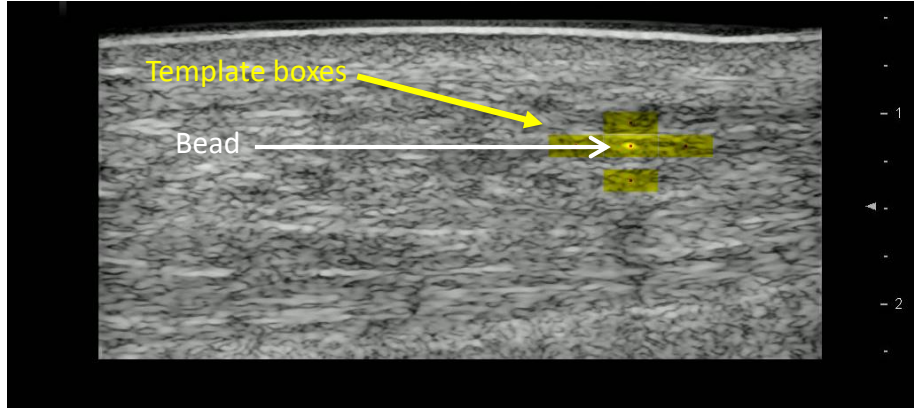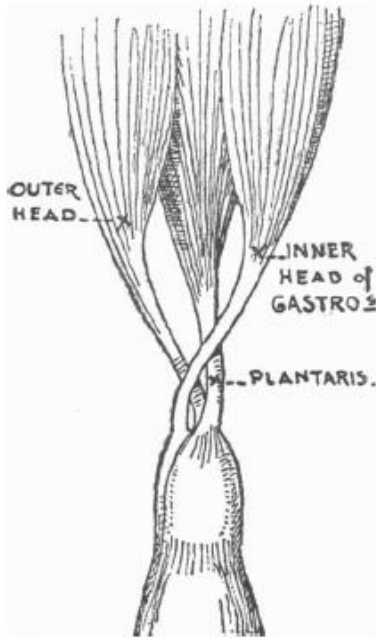
Figure 1

A

FIG. 1.—Tendo-Achillis of Beaver (*Castor canadensis*).

B

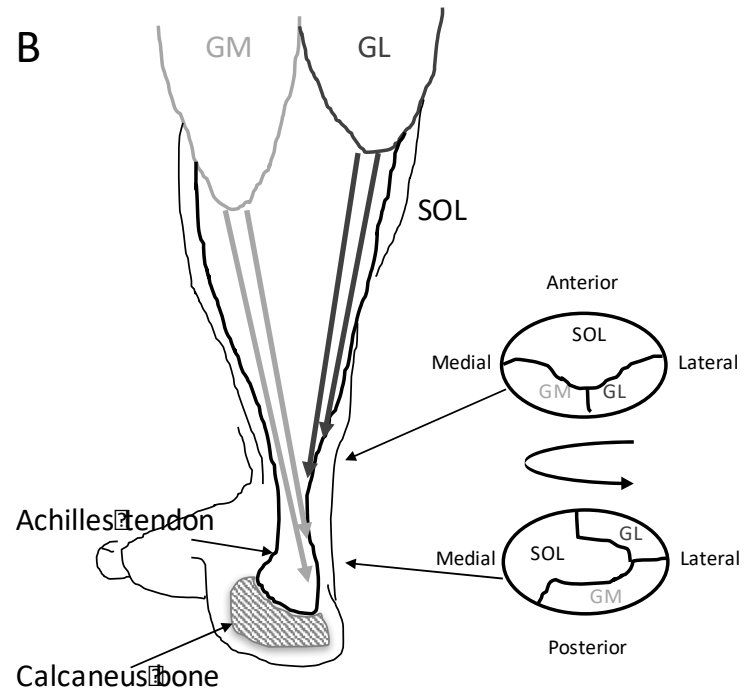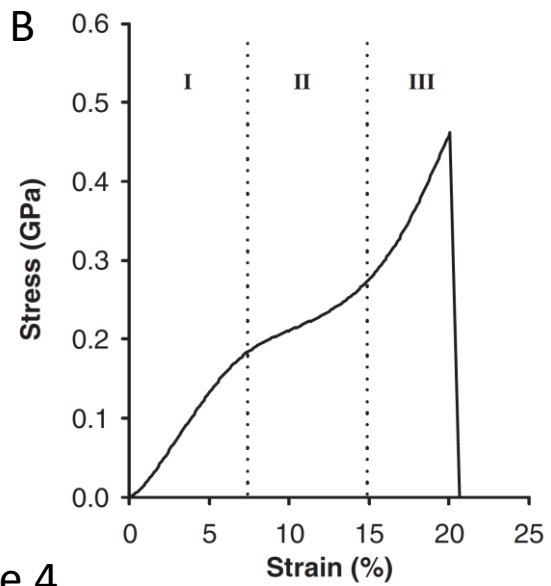

Figure 2

Figure 3

A

B

C

Figure 4