Ultrasound-based testing of tendon mechanical properties: a critical evaluation

O. R. Seynnes,1 J. Bojsen-Møller,1 K. Albracht,2 A Arndt,3 N. J. Cronin,4 T. Finni,4 and S. P. Magnusson5

1Norwegian School of Sport Sciences, Oslo, Norway; 2Institute of Biomechanics and Orthopaedics, German Sport University, Cologne, Germany; 3GH, The Swedish School of Sport and Health Sciences, Stockholm, Sweden; 4Neuromuscular Research Centre, Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland; and 5Institute of Sports Medicine, Copenhagen & Musculoskeletal Rehabilitation Research Unit, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark

Submitted 22 September 2014; accepted in final form 18 November 2014

TENDONS AND APONEUROSES TRANSMIT forces from contracting muscles to bone, but these load-bearing tissues also act as biological springs, storing and releasing elastic energy (12). This elasticity enables tendons to fulfil a number of functions related to the mechanical efficiency and effectiveness of animal and human movements (83). It follows that an accurate measurement of variables defining tendon properties (e.g., strain, stiffness, or elastic modulus) is pivotal to the understanding of the muscle-tendon unit (MTU) behavior. While mechanical testing of tendinous tissue was previously performed on animal and cadaver material, relatively recent advances in imaging techniques, such as ultrasonography, has enabled the examination of human tendon tissues under physiological conditions in vivo. In a couple of seminal reports, Fukunaga and colleagues (30, 31) first described an in vivo method to assess the displacement of human tendinous tissues during contraction. This technique, based on B-mode ultrasonography, later enabled the estimation of tendon-aponeurosis mechanical properties by combining force and the corresponding tissue deformation (e.g., Refs. 36, 58). Since then, ultrasonography has been increasingly used to test tendon properties, and this noninvasive approach enabled the demonstration of tendon plasticity in response to reduced (14, 43, 80) or increased (6, 40, 46, 82) loading. Yet the mechanical and material properties reported in various studies seem to differ considerably. For instance, differences as large as 30% can be found between measurements of patellar tendon stiffness obtained by different researchers in comparable populations and with similar methodologies (20, 86). Furthermore, Achilles tendon hysteresis values reported from humans vary between 7 and 37% compared with the ~10% reported in animal studies (26). Discrepancy is also present in the broad range of changes observed in tendon mechanical properties in response to resistance training, sometimes in spite of studies being performed by the same group (44, 50). This inconsistency currently limits our understanding of tendon function and adaptation to altered loading.

Importantly, ultrasound-based measurements are reliable (33, 84), and, in fact, the ability to detect differences or changes in tendon mechanical properties is considerable when essential precautions are taken. Nonetheless, the multitude of methodological approaches adopted by various research groups probably contributes to the large variability of reported values. The chosen procedures are often based on practical compromises relating to in vivo testing conditions (see Ref. 56 for review). However, some of these methodological choices seem simply to be due to the lack of information currently available on a complex method, which encompasses the fields of biomechanics, ultrasound scanning/imaging, digital processing, and physiology. Hence, the purpose of the present review was to assess and discuss the physiological and technical aspects associated with in vivo testing of tendon mechanical properties with ultrasonography. In doing so, our aim was to provide researchers with a systematic, qualitative analysis of ultrasound-based techniques.
METHODS DESCRIPTION

In vivo determination of tendon mechanical and material properties relies on the acquisition of the force-elongation relationship, which is used in combination with the dimensions of the tendon to obtain variables, such as stiffness, strain, stress, Young’s modulus, and hysteresis. With the exception of a few research groups using magnetic resonance imaging (MRI, e.g., Ref. 24) or radio-frequency data (24), B-mode ultrasonography is most often used to record tendon deformation during a ramped isometric plantar flexion or knee extension. Tendon force is generally calculated during isometric ramped contractions, from the externally measured joint moment, the internal moment arm, and the contribution of the coactivated antagonistic muscles (Fig. 1).

Earlier studies have considered the displacement of the proximal insertion point [e.g., gastrocnemius medialis (GM) myotendinous junction, patella apex] as a representative measurement of the total tendon elongation during contraction (48, 49, 80, 82), assuming that the motion of the distal insertion is negligible. More recent work has shown the limitations of this approach (See Incomplete scanning section below) and that the displacement of the patellar and Achilles tendon proximal insertions reflects complex contributions from tensile force, soft tissue deformation (39), and concomitant joint angular rotation that cannot be avoided, despite external fixation during isometric contractions (62, 66). To account for the effect of the joint angular rotation, the displacement of the proximal tendon insertion is typically recorded during a passive movement and subtracted from the displacement measured during muscular contractions (8, 62, 66, 81). More recently, direct measurement of whole tendon elongation was obtained by using a long ultrasound transducer (e.g., 10 cm) that enables scanning of both the proximal and distal insertions of the patellar (70) and the free Achilles (39) tendons within the same field of view. Standard transducers of 5- to 6-cm width can also be used to visualize the patella apex and the proximal part of the tibia during isometric contractions (33). In the case of the longer medial gastrocnemius tendon, two-dimensional (2D) or three-dimensional (3D) motion capture is used to simultaneously track the positions of the ultrasound transducer and the calcaneus and to measure the displacement of both tendon insertions within the same reference frame (8, 10, 53, 88).

The tendon force-elongation relation is curvilinear, consisting of an initial toe region and a linear portion. To account for random tracking errors, all recorded data points are generally fitted with a first-, second-, or third-order polynomial to determine tendon stiffness or Young’s modulus as the slope of the force-elongation and stress-strain relations, respectively (e.g., Refs. 59–61, 86) to account for random tracking errors. Some research groups have also used an exponential function (28) or a linear regression fitted exclusively to the linear part of the force-elongation relation (29, 48). The coefficient of determination usually well exceeds a value of 0.90 (33, 42, 63, 88) and may be used as a criterion for data exclusion (42). The above methods and their limitations are detailed in the following sections.

FACTORS AFFECTING IN VIVO MEASUREMENTS: METHODOLOGICAL CONSIDERATIONS

Scanning

Incomplete scanning. Ultrasound scanning of tendon deformation under isometric contractions has often been, and still is, restricted by narrow fields of view (relative to the length of patellar and Achilles tendons) and anatomical constraints (i.e., joint configuration or movement hindering transducer fixation). These limitations are sometimes overcome by scanning the myotendinous junction only, ensuring stable fixation of the transducer and, importantly, assuming that the movement of distal structures on which tendons insert is negligible during “isometric” contractions (64, 82). However, a few studies have
now demonstrated that this is not the case, notably due to the action of synergist and antagonist muscles about the same joint and because of soft tissue deformation. In fact, it seems that as much as 45% of the patellar tendon elongation and 35% of the gastrocnemius tendon elongation may be accounted for by movements of the tibia and calcaneus bones, respectively (33, 57). In the case of the free Achilles tendon, the displacement of the calcaneal insertion contributes 54–71% of the myotendinous junction displacement (39, 87), owing to the relatively short length of this tendon (~55 mm; Ref. 15). These results highlight the necessity of scanning both the proximal and distal ends of the tendon by using longer transducers (patellar or free Achilles tendon) or, in the case of longer tendons, by using an additional ultrasonic apparatus (e.g., gastrocnemius tendon).

**2D scanning of 3D deformation.** In vivo, the shape and elongation of tendinous structures is encompassed in a 3D space. First, the line of action of certain tendons does not follow a linear path (e.g., Refs. 7, 87). Disregarding the 3D conformation of tendon introduces a systematic underestimation of tendon length and thus an overestimation of length changes. Furthermore, the tendon line of action often shifts away from its original position during muscular contraction. For instance, the frontal orientation of the patellar tendon changes during quadriceps contractions (2) as a result of the medio-lateral orientation of the patella-femoral groove. Such changes are unaccounted for during ultrasonographic 2D scanning in the sagittal plane. Hence, nonoptimal positioning of the transducer relative to the patella apex and to the mean tendon line of action may lead to an over- or underestimation of deformation, resulting from the movement of the patellar apex across the ultrasound beam (Fig. 2).

It should be noted that this limitation seems less important in some cases where, for instance, the tendon origin is less recessed in the plane orthogonal to the ultrasound beam. A recent study using freehand 3D ultrasound scanning demonstrated that, despite a significant transverse strain, longitudinal strain was similar across the myotendinous junctions of the GM and gastrocnemius lateralis (23). Nevertheless, as noted by these authors, the transverse strain of the gastrocnemius tendon likely leads to some underestimate of the longitudinal strain of this tendon and of the free Achilles tendon.

Current 2D scanning methods to capture the deformation of tendinous structures are confined to a single longitudinal axis. However, when the ultrasound transducer is moved along the imaged tissues (and the transducer position is tracked), a 3D image can be created by reconstruction of the multiple 2D images. This freehand 3D ultrasound technique can overcome 2D limitations and yield important information regarding tendon deformation in several planes (23, 52, 71). Yet 3D ultrasonography requires relatively long scanning times across several planes, prohibiting muscle-tendon deformation during data acquisition and restricting studies to comparisons of still images in a resting state or during constant force contractions up to 60–70% of maximum voluntary contraction (71, 79). The development of ultrasound devices capable of capturing 3D images without displacement of the transducer seems conditional on the dissemination of this technique to studies requiring higher frequencies of acquisition.

**Tracking**

Influence of muscular contraction on tracked features. Some of the disparity in the methods found in the literature is linked to the difficulty of tracking anatomical features undergoing geometrical transformations in 3D space during loading. Such transformations occur as a result of muscular contraction, via changes in whole muscle conformation (e.g., Ref. 34) or at myotendinous junctions, with differential morphological changes between the superficial and deeper aponeuroses (e.g., gastrocnemius, Achilles, and tibialis anterior tendons). They also occur at osteotendinous junctions with rotations of the bones (e.g., patella, tibia, and calcaneus). The latter is usually due to additional passive and active forces exerted around the joint. These forces result in changes in external joint angle, despite the strapping used to fix limb segments. If overlooked, these changes can lead to an overestimation of tendon elongation (62). Thus changes in joint angle during isometric contractions are now routinely monitored by researchers, and artifactual tendon displacement can be subtracted from the measured elongation. It should be noted, however, that this precaution is not always sufficient. In the case of isometric plantar flexions, changes in ankle joint external angle are associated with changes in foot arch angle, resulting in further antero-posterior rotation of the calcaneus (38, 57).

Most current methods of estimating tendon elongation rely on the researcher’s ability to track point coordinates located near insertion sites and to calculate the distance between these points. Hence, the position of the point of interest relative to the axes of rotation of scanned structures (e.g., tibial plateau or calcaneus) influences the accuracy with which the linear tendon deformation is measured (Fig. 3). Furthermore, the con-

![Fig. 2. Difference between the axis of the patella apex displacement (dashed line) and the axis of the tibia diaphysis (solid line). The lateral and medial sides of this frontal view of the knee are labeled “L” and “M”, respectively. The figure illustrates the error in measurement of patellar displacement that can arise when the ultrasound transducer is misaligned.]
sis of this positioning across multiple analyses will affect inter- and intraobserver reliability.

**Anatomical references.** To improve the reliability of the measured deformation, some authors have tracked alternative anatomical references than the myotendinous junction sensu stricto. For example, studies on combined GM and Achilles tendons (61, 62, 80, 88), sometimes with a portion of the GM deeper aponeurosis (6), are far more frequent than reports about the free Achilles tendon. Similarly, others have estimated the elongation of the vastus lateralis deeper aponeurosis relative to the patellar tendon distal insertion (7, 15, 43) instead of the elongation of this tendon alone. In all cases, these alternative references are muscle fascicles or tendon insertions easier to scan and track in 2D than other anatomical landmarks during isometric contractions. For example, the aponeurotic insertions of vastus lateralis muscle fascicles can be scanned more easily than the limited surface covering the patellar tendon. Scanning is also easier for the Achilles tendon insertion to the GM than to the soleus, the latter being overcast by a large portion of the tendon itself. In all cases, scanning areas with multiple aponeurotic insertions of neighboring fascicles eases the tracking process by offering several alternative features to track.

While these strategies produce satisfactory accuracy and reliability (61, 62), they reflect the elongation of more complex structures than single tendons, and their interpretation is arguably limited to this context.

**Tracking techniques.** Historically, ultrasonograms of tendon (or aponeurosis) elongation were analyzed on ultrasound machines or from printouts, via measurements of distance between features of interest (31). This procedure was cumbersome and exposed to a number of problems associated with the multiplicity of manual analysis steps. In the early 2000s, authors started to analyze digitized images using dedicated software, such as NIH image (National Institute of Health, Bethesda, MD) and later ImageJ (Rasband, W.S., National Institutes of Health, Bethesda, MD), by manually measuring features displacement (51, 54). This technique is probably the most widespread currently, for it enables a relatively high reliability without any specific knowledge of digital image processing.

Yet point tracking throughout a sequence of images is not exempt from limitations. Slight movements of the transducer (e.g., resulting from changes in joint configuration or muscle conformation) often occur during in vivo recordings, influencing the gray-scale pattern of recorded images. Manual tracking heavily relies on the consistency of these patterns delineating small features. In addition, the pointing task consisting in matching particular features with a single digital point is influenced by a number of factors inherent to human-computer interaction. A few research groups have addressed these limitations by borrowing an alternative tracking approach from robotic vision (21, 63). Indeed, using optical flow algorithms (e.g., derivate of Lucas-Kanade method) to automatize tracking presents the advantages of 1) reducing human-computer interaction, and 2) improving measurement quality, through the analysis of all pixel gradients within a region of interest.

Provided that images brightness is consistent, the accuracy and reliability of flow analysis to track small features displacement are reportedly higher (e.g., maximum error < 2%, and within-trial coefficient of variation = 0.6~1.3% for the deformation of GM tendon; Ref. 61) than with manual or semiautomatic methods (e.g., coefficient of variation = 4.1% for the deformation of GM tendon; Ref. 27).

Another automated method, speckle tracking, presents similar benefits to flow analysis, without the necessity to rely on gross anatomical structures for tracking, enabling the calculation of displacement and strain within a single anatomical structure. Ultrasound speckle tracking is based on tracking of unique patterns created by interference of reflected ultrasound between a series of frames, which enables tracking of points in, for example, the distal free Achilles tendon (9). A validation study using speckle tracking on the human flexor digitorum superficialis tendon (41) described a relative error in displacement of 1.6% compared with displacement estimated from tracking of the musculo-tendinous junction. Other studies have reported high correlations (r = 0.64 to r = 0.997) between speckle tracking results in tendon tissue and model-based calculations (75, 94). The study by Arndt et al. (9) also indicated a high reliability for displacement measurements in the Achilles tendon. However, algorithms originally developed for assessment of strain in the myocardium may not be accurate in assessing strain in tendon tissue, and further validation studies are required (65).

A further technique, which has received recent attention in research attempting to quantify internal tendon dynamics, is ultrasound elastography. This technique also uses speckle patterns within the tissue of interest. Phase information in the ultrasound radio-frequency data is used to track the displacement of such patterns in the direction of the ultrasound beam (17, 72). Similar to speckle tracking, elastography has been applied to tendon tissue for determining displacement and strain (17), and good validity and correlations compared with digital image correlation have been reported (18).
Automated tracking methods of tendon deformation seem superior to manual methods. Yet, unless they are included in a proprietary software package of ultrasound manufacturers, they require a certain mathematical/computing expertise and equally rely on scanning accuracy of specific features during tendon deformation (see limitations in the above section). The publication of algorithms written for (semi-) automatic tracking or the use of open source algorithms (21, 63) seems critical to allow a full appraisal of published material and to enhance the quality of data analysis.

Estimation of Tendon Force

Unlike in vitro experiments or invasive procedures, the force exerted during in vivo tendon testing can only be estimated. Calculations depend on a few assumptions, introducing a certain amount of error in the assessment of tendon mechanical properties. Additionally, the various methodological approaches chosen for the quantification of tendon force in vivo underpins some of the discrepancy between laboratories.

Tendon force calculations are based on resultant joint moments predominantly produced by the muscles to which the tendon of interest is inserted. The biomechanical analysis is reduced to a single agonist force vector in line with the tendon direction and, in many cases, a single antagonist force vector opposite to the agonist one. The magnitude of the latter is typically estimated by assuming linearity between electromyographic activity and isometric moment production. Although such a relation has been observed for some muscles (4, 13, 36), it is currently unknown whether it remains similar when muscles act as antagonists and, in the case of muscle groups (e.g., hamstrings or plantar flexors), whether the electromyographic activity of a single muscle is representative of its synergists. Nevertheless, depending on the methodology and the considered muscle groups, antagonist cocontractions seem to generate a moment somewhere between 10 and 30% of the measured joint moment (1, 59). A few research groups have deemed the error originating from such an estimation to be larger than when considering agonist moment alone and have thus dismissed antagonist coactivation when calculating tendon force (e.g., Ref. 86).

An additional challenge for the estimation of tendon force resides in the measurement of the moment arm length. Largely determined by equipment availability, the choice of methods typically includes ultrasound/MRI-based techniques, such as the tendon excursion method (5) and the center of rotation method (58), external measurements or estimations based on normative data (67). While various degrees of convergent validity can be inferred, all of these methods are more or less influenced by certain limitations. For example, methods relying on normative data ignore interindividual anatomical differences, and the fact that moment arms do not always scale to other anatomical features (56). In addition, some authors have assessed moment arm values from the resting MTU, at a given joint angle, overlooking the influence of joint angle specificity or muscle contraction on this variable (56). Like all measurements performed in vivo, the estimation of moment arm length is bound to a number of assumptions that do not invalidate the use of these techniques. However, the inaccuracy stemming from these assumptions hinders the precise assessment of force and potentially influences the interindividual variability and external validity of calculated tendon mechanical properties. The use of different methods induces differences as large as 40–50% in patellar tendon moment arm length (92) at a knee joint angle of 90° (typically used to test tendon properties). To illustrate the consequences of this problem, a 40% difference in estimated moment arm length would result in a 67% difference in calculated force.

Methodological discrepancies are also frequently found in the conversion of the recorded force/moment (raw or corrected for antagonistic coactivation) into tendon force.

Unlike in vitro conditions, MTUs are never isolated in vivo. First, forces may be shared among branches of some tendons (e.g., Achilles tendon; Ref. 88), between tendons and other structures such as retinacula (78), or spatially dispersed into compressive or shearing components (69). This complexity is illustrated in the difference between patellar tendon and quadriceps tendon forces, or in the fact that Achilles tendon force cannot be attributed to the force generated by the gastrocnemius muscle alone, nor can it be mistaken for the force produced by the whole triceps surae. The various limitations of existing methods of measuring tendon force (27) probably contribute to the discrepancy between in vivo and in vitro methods, although similar and additional limitations (e.g., specimen clamping) also pertain to the latter. Furthermore, a consensus is still lacking on the assessment of tendon force in vivo (Refs. 60 vs. 53), preventing direct comparison of calculated mechanical properties between studies. For example, some authors have used the estimated force production of the GM only (corresponding to forces between 500 and 900 N; Refs. 21, 53, 78) to calculate gastrocnemius tendon properties, whereas others have used the whole plantar flexion force (1,400 to 4,400 N; Refs. 2, 31, 60). This methodological choice results in proportionally large differences in calculated tendon properties and limits comparisons between studies.

Signals Synchronization

Image acquisition should aim for high temporal and spatial resolution, and absence of image lag when collecting other signals synchronously. The possibility for errors in synchronization of signals from different sources due to computer processing time is often neglected. Furthermore, in ultrasonography, the rate at which images are generated is usually much lower than the force sampling frequency, increasing the potential occurrence of synchronization errors.

The effect of desynchronization of tendon elongation obtained from ultrasound images and force on tendon stiffness and hysteresis was recently illustrated by Finni et al. (26). Purposely offsetting the tendon elongation data collection by 10 ms caused hysteresis to increase from 6 to 15%, although the shift had a much smaller effect on stiffness. In practice, with an ultrasound sampling frequency of 50 Hz, the effect of desynchronization is 20 ms/frame (26). If uncertainty in synchronization can be considered a random source of error, the problem may be avoided by averaging a sufficient number of trials (84).

FACTORS AFFECTING IN VIVO MEASUREMENTS: PHYSIOLOGICAL CONSIDERATIONS

In addition to the technical challenges described above, a number of physiological factors impart another level of com-
Complexity to tendon testing, contributing to the variety of methodological choices and to discrepant observations.

**Conditioning and Rate-dependent Mechanics**

An important, often overlooked, physiological consideration is the viscous nature of tendons. This characteristic means that tendons display time-dependent properties, such as stress-relaxation and creep. Under a constant strain, stress decreases (stress-relaxation) and when a tensile stress is imposed continuously or cyclically, strain tends to increase over time (creep). One consequence of these phenomena is the history dependence of tendons, whereby mechanical properties are not stable unless testing is immediately preceded by at least five conditioning contractions (55, 84). Conditioning, which in some cases accounts for as much as 45% of tendon elongation (55), is currently missing from the methodological description of most studies including in vivo tendon testing.

Rate-dependent mechanics have other methodological implications. Typical instructions given to subjects performing an isometric ramp contraction are to gradually increase exertion until maximal moment, within a given time (4–10 s; Refs. 14, 84). However, such an approach implies that loading rate will vary between stronger and weaker subjects, and between studies using different contraction times. It also means that, without visual feedback, loading rate will randomly differ between trials, between subjects, and even within trials (42, 73). The consequences of this lack of control have been illustrated in some animal studies, showing the influence of strain/loading rate on the measurement of tendon mechanical properties (19, 68). In humans, a few studies, yet not all (49, 76), have confirmed the rate dependence of tendon mechanics in vivo (42, 74, 91) and in vitro (93); nonetheless, this property remains largely overlooked. A thorough control of loading rate per se, as opposed to the contraction duration only, seems required to improve the reliability of in vivo tendon measurements (42). Moreover, comparisons between studies have routinely been discussed under the assumption that mechanical properties are comparable within the elastic region of the force-elongation curve, regardless of variations in contraction time. Taken together, the rate dependency of tendon properties and the natural pace at which human tendons are loaded in daily activity suggest that in vivo tendon properties should generally be tested within similar ranges of loading rates, as fast as methodological conditions allow. In turn, particular attention should be paid during familiarization sessions, to ensure that subjects are able to increase or decrease joint moment linearly at a target rate.

**Nonlinear Force-Elongation**

Tendon viscoelastic properties, and in particular the nonlinear force-elongation behavior of this tissue, is connected to another methodological challenge: the standardization of stiffness calculation. This parameter is typically obtained as the slope of the tendon force-elongation curve over an interval ranging from 10 to 50% of the maximal force (i.e., between 50 and 100% or between 90 and 100% of maximal force; Refs. 5, 39). Such variability in the choice of calculation is essentially based on a dual necessity. On the one hand, the slope of the force-elongation curve is determined by a polynomial function. Considering a broader range of the fitted curve is a sensible way to decrease the influence of biases associated with scanning and analysis techniques. On the other hand, in vivo measures rely on maximal voluntary contractions, which produce stress levels far lower than ultimate values. Considering a small force range in the upper region of the voluntary force-elongation curve (e.g., 10%) increases the chances of obtaining a tendon stiffness value from the elastic region (i.e., beyond the toe region). Theoretically, both approaches are appropriate since measurements are performed within the linear portion of the curve: the conservative threshold of 30 MPa beyond which

| Table 1. **Recommended methodological approach when assessing tendon properties in vivo using ultrasonography** |
|---------------------------------------------------------------|---------------------------------------------------------------|
| **Possible Sources of Error** | **Preferred Approach** |
| Scanning | Scanning of both tendon insertions, within the same field of view when possible. |
| Incomplete scanning | In addition to tendon location and direction, adjustment of transducer position to account for the 3D nature of tendon deformation. |
| 2D scanning of 3D deformation |  |
| Tracking | Account for changes in joint angles occurring during isometric contractions, to ensure consistency of the position of the tracked region relative to anatomical references. The tracked regions should be positioned around or within the tendon insertion. |
| Stability of experimental setup |  |
| Anatomical references | The properties of tendons or tendon portions free from attachments (i.e., to adjacent aponeuroses) may be obtained more accurately when obtained via tracking of their insertions than via tracking of related anatomical structures (i.e., aponeuroses). |
| Tracking techniques | Automated tracking. Generally, any method reducing manual intervention and experimenter bias. |
| Force estimation | Measure/calculate moment arms individually. Train subjects to reduce cocontractions. |
| Signal synchronization | Data acquisition centralized into a single software. Use higher frequencies of acquisition or a frame grabber. |
| Conditioning and rate-dependent mechanics | Perform at least 5 consecutive contractions at 50% of MVC or more before initiating ramp contraction. Loading rate per se should be controlled. Faster contractions can be performed more reliably and likely reflect mechanical properties closer to daily function. In any case, sufficient familiarization is necessary to ensure that subjects are able to increase or decrease moment production linearly at a target rate. |
| Nonlinear force-elongation relation | Calculate stiffness and elastic modulus within highest force/stress intervals above 30 MPa. |
| Standardization of tendon resting length | Standardize joint configuration to set the length of the MTU(s) connected to the tested tendon close to “anatomical” values. |

Table shows the authors’ consensual recommendations for a selected number of issues related to in vivo testing of tendon mechanical properties. 2D, two-dimensional; 3D, three-dimensional; MVC, maximum voluntary contraction; MTU, muscle-tendon unit.
mammalian tendons elongate near linearly (11, 77) is usually exceeded (30–50 MPa in most cases; Refs. 5, 19, 39, 44, 60, 80, 83) in human studies involving voluntary contractions. However, the stress interval over which tendon properties are calculated may overlap the 30-MPa toe limit (for a maximal stress of 40 MPa, a range between 20 and 40 MPa must be used to calculate Young’s modulus between 50 and 100% of maximal stress). Consistent with this observation, studies on both gastrocnemius (54) and patellar (82) tendons indicate that, in vivo, the relation between stiffness and force is asymptotic. These data suggest that the linear region of the tendon force-elongation relationship is only reached in the upper region of the voluntary force range, and measuring mechanical/material properties over a broader range may induce errors. A notable exception to this limitation can be found in cases where tendon properties are assessed with an experimental setup inducing an initial tension affecting tendon length before the onset of muscle contraction (e.g., Ref. 74). In such cases, the setup removes the toe region from the tendon’s force-elongation curve.

Standardization of Tendon Resting Length

In contrast to in vitro testing, where the length of specimens can easily be standardized via measurements of collagen crimp patterns, there is currently no method to characterize tendon resting length in vivo. Joint configurations are usually chosen to enable stable testing conditions and as compromises between baseline stress and tendon slackness. Current, informal conventions on joint configurations offer a reliable alternative, but small differences between studies and/or individuals may affect the calculations of tendon mechanical properties.

For the gastrocnemius tendon, authors have used a wide range of knee and ankle angle combinations, some of which (Refs. 46 vs. 21) lead to 5% differences in the length of the MTU (estimation based on Ref. 33). For the patellar tendon, the angle of knee joint flexion has conventionally been held at 90° (40, 82, 85), with a few exceptions (e.g., 80°; Refs. 42, 47). Different combinations of hip and knee joint angles found in published reports seemingly yield smaller differences in estimated MTU length in this case (~1% for rectus femoris MTU length, estimations based on Ref. 33). Since methodological resources are currently lacking to accurately determine or standardize tendon test length, a standardized joint configuration remains the best way of controlling tendon length before muscle contraction. Yet differences between studies in this parameter, and interindividual differences in the joint angle corresponding to the onset of tendon loading, are likely accountable for some of the variability of tendon deformation measurements. Moreover, some tendons from biarticular MTUs are connected to the tendons of mono-articular MTUs (e.g., GM tendon branching out from the free Achilles tendon). This anatomical complexity suggests that matching the length of biarticular MTUs via different joint configurations probably induces regional differences in strain.

CONCLUSION

Until recently, our understanding of the biomechanics of tendon has largely been limited to studies on animal and cadaver tissue. However, with the advent of ultrasound-based technology, coupled with estimation of force, it is now possible to obtain biomechanical information on human tendon properties in vivo. This has prompted numerous studies of human tendon behavior and responses to various conditions and long-term interventions over the last 2 decades. However, the available values in the literature vary considerably, and numerous ultrasound-based methodological approaches have been used. Herein we have outlined some of the technical challenges and solutions that should be considered using the ultrasound method (Table 1).

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Tron Krosshaug and Oliver Faul for help with Fig. 1.

GRANTS

This review was supported by the Nordic Muscle Tendon Network.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES

Synthesis Review


60. Magnusson SP, Hansen P, Aagaard P, Bro nd J, Dyhre-Poulsen P, Kjaer M. Differential strain patterns of the human...