

Mechanical Properties of Aponeurosis and Tendon of the Cat Soleus Muscle During Whole-Muscle Isometric Contractions

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ABSTRACT Recent studies have suggested that the mechanical properties of aponeurosis are not similar to the properties of external tendon. In the present study, the lengths of aponeurosis, tendon, and muscle fascicles were recorded individually, using piezoelectric crystals attached to the surface of each structure during isometric contractions in the cat soleus muscle. We used a surgical microscope to observe the surface of the aponeurosis, which revealed a confounding effect on measures of aponeurosis length due to sliding of a thin layer of epimysium over the proximal aponeurosis. After correcting for this artifact, the stiffness computed for aponeurosis was similar to tendon, with both increasing from around $8 F_0/L_c$ (F_0 is maximum isometric force and L_c is tissue length) at $0.1 F_0$ to $30 F_0/L_c$ at forces greater than $0.4 F_0$. At low force levels only ($0.1 F_0$), aponeurotic stiffness increased somewhat as fascicle length increased. There was a gradient in the thickness of the aponeurosis along its length: its thickness was minimal at the proximal end and maximal at the distal end, where it converged to form the external tendon. This gradient in thickness appeared to match the gradient in tension transmitted along this structure. We conclude that the specific mechanical properties of aponeurosis are similar to those of tendon. © 1995 Wiley-Liss, Inc.

The contractile components of muscle exert forces on the relatively rigid skeleton via in-series connective tissue. The mechanical arrangements and properties of these connective tissues may have important effects on the dynamics and energetics of the relationship between muscle fascicles and skeleton. For example, spring-like compliance in the connective tissue may absorb and release elastic strain energy (Alexander and Vernon, '75; Cavagna, '77) and may distort the relationship between the overall length of a muscle and the proprioceptive feedback from length sensors such as muscle spindles (Rack and Westbury, '84; Hoffer et al., '89; Griffiths, '91, but see Elek et al., '90). The magnitude of these effects usually depends on the specific morphometry of the muscle, and extrapolation to other muscles requires an explicit or implicit mathematical description. However, the selection of the qualitative form and particular quantitative values for the components of the model of whole muscle is hampered by the paucity of experimental data

from muscles representing a wide range of musculotendinous architectures.

An alternative to the study of whole muscles is to develop models of particular muscles based on general mechanical functions provided by the various individual tissues of which they are composed: contractile components, parallel-elastic components, series-elastic components and the mechanical coupling among them. The mechanical functions that reside wholly within the external tendon or individual muscle fibers are amenable to study in isolated preparations of those tissues (e.g., Ker, '81 and Edman, '88, respectively). However, the muscle fibers of many muscles are attached not to tendon or bone, but rather to sheets of internal and/or external connective tissue called aponeuroses. The mechanical properties of an extensive aponeurosis may dominate the series-

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elastic and mechanical coupling functions, but these properties are not well understood.

The intimate and distributed connections between an aponeurosis and terminating muscle fibers make it difficult to estimate its mechanical properties directly. Techniques such as photogrammetry have been employed only recently to record length changes of connective tissue during changes in muscle force (Huijing and Ettema, '88/89; Ettema and Huijing, '89; Lieber et al., '91; Zuurbier et al. '94). Based on such data, Lieber et al. ('91) suggest that aponeurosis and tendon have very different mechanical properties: aponeurosis was found to be four times more compliant than tendon. Recently Ettema and Huijing ('89) claimed to have shown that the length of an aponeurosis is dependent not only on muscle force, but also on fascicle length. These studies suggest that the organization of an aponeurosis is complex and that its mechanical properties are not similar to external tendon.

In the present study multiple piezoelectric crystals were attached to the surface of the cat soleus muscle. These provided direct measurement of the length of the aponeurosis and tendon during isometric contractions performed at different whole-muscle lengths. These recordings were used to estimate the stiffness properties of the tendon and aponeurosis. In addition, anatomical techniques were used to relate the structure of the aponeurosis to its mechanical properties.

MATERIALS AND METHODS

Data collection

Experiments were carried out on adult cats (2.75–4.7 kg; either sex) anesthetized initially with 35 mg/kg of sodium pentobarbital IP with supplemental doses IV as needed to suppress withdrawal reflex. An incision was made along the posterior surface of the right leg from the calcaneum to the popliteal fossa. The popliteal fat pad was removed and the plantaris and gastrocnemius muscles were resected. A tri- or bipolar nerve cuff was placed around the tibial nerve. Nerve stimulation was elicited by a biphasic constant current pulse with a duration of 0.2 ms and an amplitude of four times the current to elicit a just-visible contraction. The soleus tendon and a small piece of the posterior calcaneus were removed from the foot and attached to a force transducer (linear up to 55 N), which was attached to a stepping motor. A computer-controlled data-acquisition system was used to deliver prepro-

grammed sequences of electrical stimuli to the nerve entering soleus (50 Hz for 400 ms) while controlling the position and velocity of a step-motor attached in-series to the muscle. Whole muscle isometric contractions were recorded at 16 lengths that evenly spanned the anatomical range of motion of soleus. The program recorded the force generated by the muscle and the position of the motor, which was calibrated in terms of whole-muscle length. The program also recorded the length of individual portions of the muscle (fascicle, aponeurosis and tendon) using piezoelectric crystals (Sonomicrometer 120; Triton Technology) attached to the surface of the muscle. The crystals transmitted ultrasonic pulses through a pool of paraffin oil; the pool was created by tethering the skin flaps to a metal ring. Each ultrasonic pulse was transmitted by one crystal and received by another; the transit time was converted into a measure of length. The transmission of ultrasonic pulses through the paraffin oil, rather than directly through the muscle tissue (i.e., Griffiths, '91; Hoffer et al., '89) negates the conflicting effect of muscle force on the velocity of the ultrasonic signals (see Hatta et al., '88; Caputi et al., '92). Some preliminary experiments were performed in which the crystals were attached to a Silastic sheet (Reinforced Silastic Sheeting, Dow Corning) that was sutured to the muscle surface. In subsequent experiments, the crystals were attached to the surface of the muscle using cyanoacrylate adhesive (Accu-Flo Crazy Glue, Lepage). Crystals attached by the adhesive were found to remain secure for long periods of time, although constant monitoring was required in order to ensure the stability and alignment of the crystals and thus the quality of the output signals. Although the glue may tend to augment the stiffness properties of the aponeurosis, the magnitude would be minimal since the size of the drop of glue was small relative to the distance traversed between crystals (approx. 1:15).

Crystals were positioned initially at the proximal and distal ends of the aponeurosis to record the entire length of this structure. The distal end of the aponeurosis was defined as the most distal point of muscle-fiber attachment. Most of the aponeurosis appears as longitudinal white strands of connective tissue that converge to form the external tendon. However, the proximal end of the aponeurosis is less evident; its border can best be visualized at long muscle lengths when the

perimeter of the aponeurosis can be seen as a change in light reflectance due to the angular change between the surface of the fascicles and aponeurosis.

The length of the entire aponeurosis was recorded by one set of crystals in the first few experiments. In later experiments, a crystal was positioned in the middle of the aponeurosis, and the lengths of the distal and proximal portions of the aponeurosis were recorded simultaneously. In the last three experiments, crystals were also attached to the medial and lateral edges of the aponeurosis so that the width of this structure could be recorded simultaneously with its length.

The length of the entire tendon could not be recorded because the crystals must transmit their ultrasonic pulses through some medium, in this case, a paraffin oil pool. The distal end of the tendon, which was attached to the clamp, was above the paraffin pool for contractions at all but the shortest of whole-muscle lengths. Therefore, the crystals were positioned at the most proximal end of the external tendon and at a position 1.0–1.5 cm more distally. Measures of this segment of tendon were possible at most, but not all whole-muscle lengths.

Data analysis

The force-length relationships of the aponeurosis and tendon were computed during individual isometric contractions at 16 different whole-muscle lengths.

Stiffness is a measure of the relationship between the change in force transmitted by a structure and its corresponding change in length. For the contractions recorded in the present study, the simplest method to estimate stiffness would be to compare the change in muscle force and tissue length between adjacent data-collection sample periods. However, the nonlinear rise and fall in muscle force during tetanic contractions result in large and rapid changes in length at low force levels and very slow changes at higher force levels when peak muscle force was approached. Therefore, the variability in the estimates of stiffness would increase at high force levels because the change in force and length were small between adjacent sample periods. In the present study, stiffness was estimated at specific force levels equal to increments of $0.1 F_0$ (where F_0 is the maximal isometric force generated at the optimal fascicle length, L_0). Each estimate of stiffness was based on interpolation from tissue lengths recorded at force levels that were

$0.1 F_0$ above and below the desired force level (Fig. 1). Stiffness estimates were normalized to F_0 for each preparation and to L_c , the length of the recorded tissue at slack length and zero muscle force, in order to define a dimensionless term called the "specific stiffness" of the tissue. To determine the stiffness of a particular muscle's connective tissue, the dimensionless specific stiffness would be multiplied by that muscle's F_0 (typically estimated from its physiological cross-sectional area in cm^2 and the specific tension of muscle in N/cm^2) and divided by its L_c (in cm) resulting in N/cm .

Anatomical observations

The superficial surface of the soleus muscle was examined, using a surgical microscope (Ome Olympus), in four cats. The gastrocnemii were resected, and the surface of the aponeurosis and the epimysium of the soleus were observed under static and dynamic conditions.

The right soleus muscles of two cats were dissected and fixed in 10% formalin for 24 hr. The mass of each muscle was recorded along with the length of the aponeurosis. The muscles were then cut along the midline of the muscle and orthogonal to the surface of the aponeurosis. Each half was trimmed just above the proximal end and just below the distal end of the aponeurosis. The remaining portions were divided into three sections and each section was embedded in a mixture of paraffin and plastic polymers (Paraplast, Sherwood Medical). A single $5\text{-}\mu\text{m}$ section was cut from each block orthogonal to the

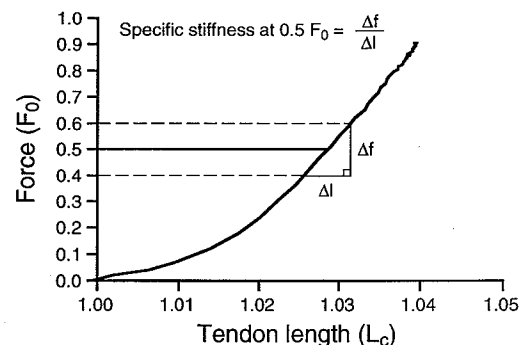


Fig. 1. *Felis catus*. The specific stiffness of connective tissue was estimated at increments of $0.1 F_0$ from the whole-muscle isometric contractions. Each estimate was based on the tissue lengths recorded at force levels that were $0.1 F_0$ above and below the desired level.

surface of the aponeurosis and stained with Gomori's trichrome to highlight the connective tissue of the aponeurosis (Drury and Wallington, '80). The thickness of the aponeurosis was calculated at 1-mm intervals along its surface from each section, using a microscope graticule at 250 power (Dialux 20, Leitz Wetzler). Although shrinkage of the connective tissue was not accounted for in this analysis, we were interested primarily in the relative thickness along the extent of this tissue.

RESULTS

The force-length properties of the connective tissue in the soleus were analyzed fully in five cats. The lengths of the aponeurosis and tendon were 4.4 ± 0.6 cm and 2.7 ± 0.3 cm, respectively, at slack length under passive conditions. Therefore, the nominal fascicle length of the soleus (3.8 ± 0.6 cm) is approximately one-half the length of its in-series connective tissue.

The measurement of the relationship between the force generated by the muscle and the length of any portion of the muscle depended substantially on the quality of the attachment of the crystals to the muscle surface. Figure 2A displays the recorded force-length relation of the aponeurosis when the crystal was seen to be attached poorly to the underlying surface of the muscle. The recording shows a large difference in the force-length relation between the rising and falling phase of the contraction, suggesting that there was a large hysteresis between loading and unloading of the aponeurosis. Upon reat-

tachment of the crystals to the muscle surface, the large hysteresis between the rising and falling phase of isometric contractions disappeared (Fig. 2B). Similar inconsistencies between the rising and falling phases of the contractions were found during preliminary experiments in which the crystals were attached to a Silastic Sheet (Reinforced Silastic Sheeting, Dow Corning), which was sutured to the surface of the muscle. In the experiments described here, all crystals were monitored constantly to ensure that they remained attached firmly to the underlying tissue by cyanoacrylate adhesive.

Comparisons of length and computations of stiffness presented here for tendon and aponeurosis are all based on the rising phase of the contractions, which showed only small and inconsistent differences in stress-strain relationships from the falling phase. We have therefore assumed throughout that these structures act purely elastically and have negligible viscosity under physiological conditions.

Aponeurosis and tendon lengths during isometric contractions at different muscle lengths

Figure 3 illustrates the length changes in the fascicle, aponeurosis and tendon during the rising phase of isometric contractions at five different whole-muscle lengths. In general, the force-length relationship of tendon was independent of starting muscle length. However, the starting length of the aponeurosis shifted in a way that was not dependent on muscle force but rather on muscle length,

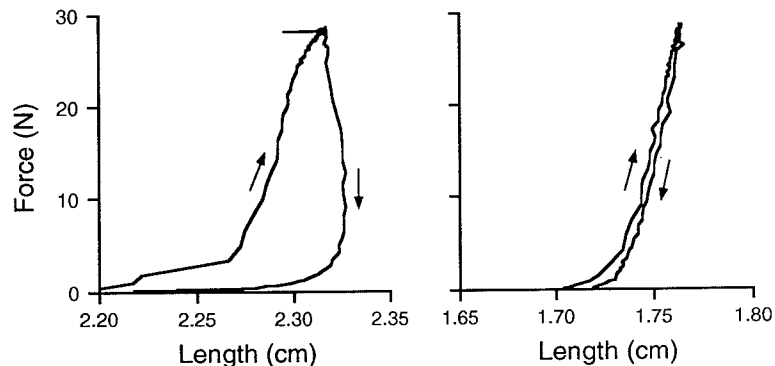


Fig. 2. *Felis catus*. The recordings of the aponeurosis were affected by the quality of the attachment of the piezoelectric crystals. **A:** A large difference in the force-length relationship for the rising and falling phase of contractions was often recorded when the crystals were

attached poorly to the underlying surface of the muscle. **B:** The hysteresis during contractions largely disappeared when the crystals were re-attached firmly to the muscle surface.

producing a family of force-length curves shown in Figure 3B. Similar shifts in the length of the aponeurosis associated with the length of the fascicles have been reported in rat medial gastrocnemius and extensor digitorum longus muscles (Huijing and Ettema, '88/89; Ettema and Huijing, '89).

In order to study the properties of the aponeurosis further, the length of the proximal and distal portions of the aponeurosis were recorded separately, as was the width across the proximal end of the aponeurosis (Fig. 4). The proximal and distal portions of the aponeurosis both increased in length as force rose during the contraction. The starting length of the proximal portion of the aponeurosis continued to shift with changes in whole-muscle length. In fact, the relative change in length increased. In contrast, the length of the distal portion of the aponeurosis did not shift with fascicle length. The dependence of aponeurosis length on fascicle length seemed to be isolated to only the most proximal end of the aponeurosis; this corresponds with the region of the aponeurosis where the strands of connective tissue diminish in size and terminate. The width of the aponeurosis increased during the rising phase of the contractions (Fig. 4D). The initial width

of the aponeurosis also increased when the fascicles shortened.

Anatomical observations of the surface of the aponeurosis

Observations of the surface of the aponeurosis using light microscopy revealed that the surface was composed of a thin layer of crimped collagen fibers, similar to the collagen in the external tendon (Diamont et al., '72; Rowe, '85). The collagen was organized into longitudinal bands approximately 200 μm wide. The width of the aponeurosis was relatively uniform along its entire extent: this dimension was maximal near its proximal end and decreased slowly distally. At its distal end, the longitudinal bands converged to form the external tendon. The relatively uniform width of the aponeurosis suggests that termination of muscle fibers and thus transmission of muscle fiber force onto the aponeurosis was relatively consistent and uniform along its longitudinal axis.

The muscle was covered with epimysium composed of a thin membrane containing collagen fibers oriented in a crossed-ply arrangement similar to the observed meshwork of collagen in the perimysium and endomysium (Rowe, '81; Purslow, '89), except

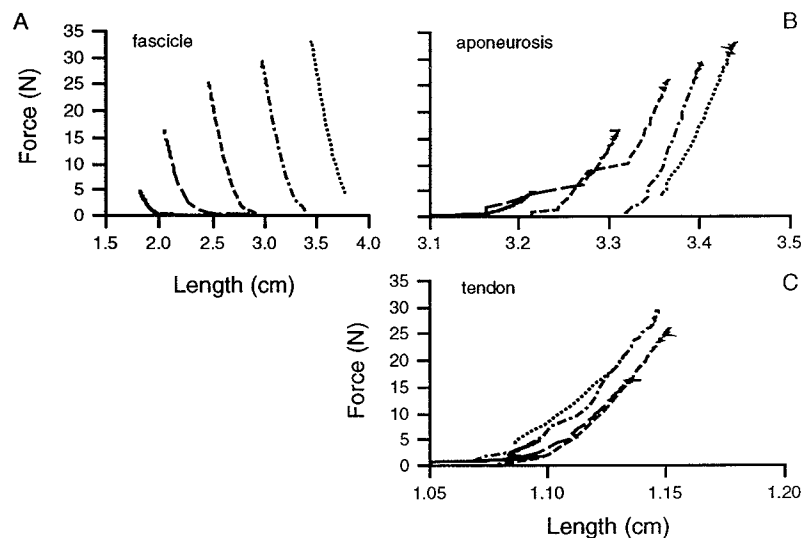


Fig. 3. *Felis catus*. The lengths of the fascicle, aponeurosis and tendon are shown during the rising phase of whole-muscle isometric contractions recorded at different muscle lengths. Measurements from the same contraction are denoted with similar line types. A: The fascicle shortened as force increased during each contraction and its length increased for contractions recorded at longer

whole-muscle lengths. B: The length of the aponeurosis increased with force during each contraction, but its starting length shifted for contractions at longer whole-muscle lengths. C: The change in length of the tendon during contractions was consistent at all whole muscle lengths.

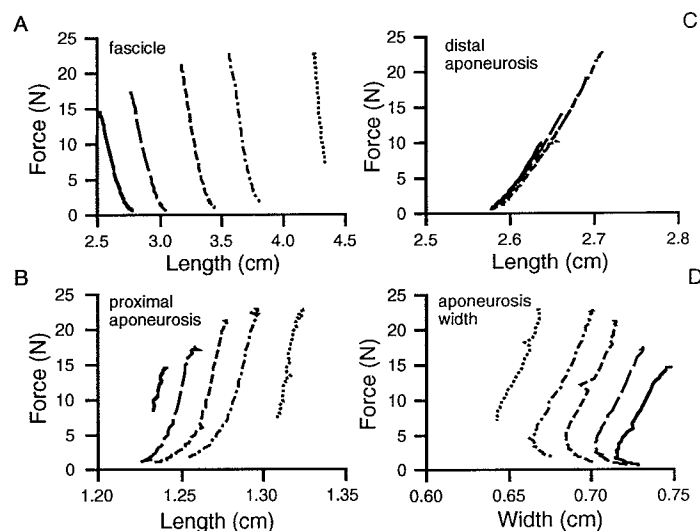


Fig. 4. *Felis catus*. The length of different portions of the aponeurosis, as well as the muscle fascicle were recorded simultaneously during the contractions (A–D). Measurements from the same contraction are denoted with similar line types. Note that the length of the proximal, but not the distal portion of the aponeurosis shifted for contractions at different whole-muscle lengths.

that it was more sparse. The epimysial collagen fibers over the fascicles were oriented symmetrically at equal angles to the long axis of the fascicles. When the fascicles were lengthened the crossed-ply was reoriented to become more aligned with the long axis of the fascicles in order to accommodate the fascicle length change. A similar re-orientation of the crossed-ply arrangement of collagen fibers has been observed for the perimysium (Purslow, '89).

The most important feature of the epimysium is its termination onto the aponeurosis. The collagen fibers of the epimysium were observed to cover the perimeter of the aponeurosis for a couple of millimeters prior to their attachment to the collagen fibers of the aponeurosis. Therefore, when the fascicles lengthened, piezoelectric crystals that were attached to the epimysium would move away from the aponeurosis due to the lengthening of the epimysium (Fig. 5). Apparent changes in aponeurosis length would be observed only at the most proximal end because the collagen fibers of the epimysium terminate only on the perimeter of the aponeurosis. Indeed, this was what was observed in the records of aponeurosis length; length changes related to fascicle length were restricted to the most proximal end of the muscle (Fig. 4). This strongly suggests that the apparent shift in

the length of the aponeurosis related to fascicle length was artifactual and created by movement of the epimysium over the surface of the aponeurosis.

The crossed-ply collagen of the epimysium along the side of the muscle was also oriented symmetrically about the longitudinal axis of the fascicles. Therefore, epimysial sliding should alter the apparent width of the aponeurosis: fascicle shortening would lead to a decrease in the apparent width of the aponeurosis. However, the apparent width of the aponeurosis was observed to increase when the fascicle shortened based on the piezoelectric crystals. This suggests that the width of the aponeurosis does increase when the fascicle shortens and may in fact increase more than was recorded.

The midline-stained sections of the aponeurosis displayed a marked variation in the thickness of the aponeurosis along its length (Fig. 6). The thickness was minimal (less than 10 μm) at its proximal end, but increased gradually at more distal positions. Near the tendon, the thickness of the aponeurosis increased more rapidly reaching 200 μm at its most distal end. This rapid increase in the aponeurosis thickness over the distal quarter of the aponeurosis coincides with a rapid narrowing of the aponeurosis surface as the longitudinal bands converge to form

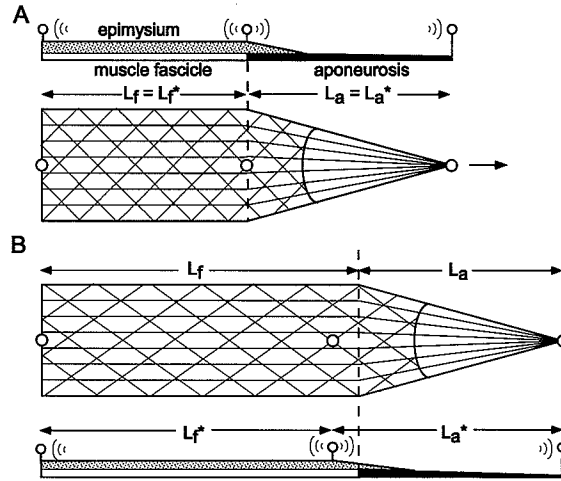


Fig. 5. *Felis catus*. Schematic lateral cross-sectional and superior views of muscle showing relationships between piezoelectric crystals used for length measurements (circles) and their mounting sites on the distal edge of the aponeurosis (right), bony origin of the muscle fascicles (left) and junction between fascicles and aponeurosis (center). Note that the central crystals are actually mounted on a slip of epimysial connective tissue (cross-

hatch) that is free to slide over the anatomical junction (dashed lines). **A:** Recorded length of aponeurosis (L_a^*) and fascicle (L_f^*) are correct when central crystal is above the junction. **B:** When the muscle lengthens, the length recorded by the crystals spanning the fascicle underestimates the true fascicle length ($L_f^* < L_f$) and the crystals over the aponeurosis erroneously record an increase in length for the aponeurosis ($L_a^* > L_a$).

the external tendon. In general, the gradient in the thickness of the aponeurosis appeared to balance the gradient in the force transmitted across this structure, supporting the notion that strain will be distributed uniformly in this structure when active tension is generated homogeneously in this muscle.

Stiffness measures

The specific stiffness of the soleus tendon is shown in Figure 7. Each value equals the stiffness averaged from all recorded contractions. Specific stiffness of the tendon was approximately $8 F_0/L_c$ at $0.1 F_0$ but rose to

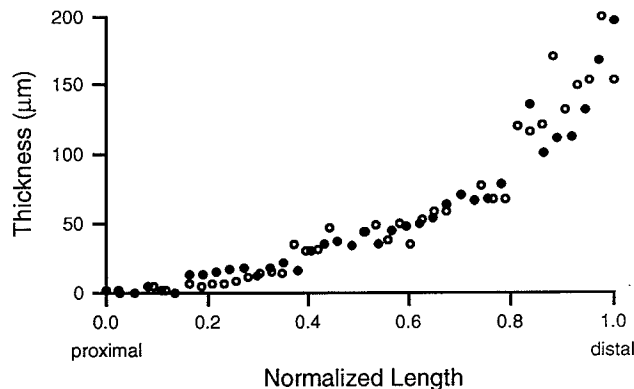


Fig. 6. *Felis catus*. The thickness of the soleus aponeurosis varies along its length. The two symbols denote the recorded thickness for two different cats (soleus muscle mass = 2.67 and 2.70 g). The abrupt fluctuations, particularly at the thick end, reflect collagen bundles crossing at a slightly oblique angle to the plane of section.

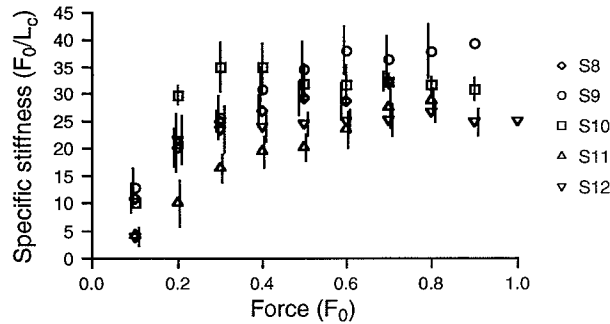


Fig. 7. *Felis catus*. The specific stiffness of the tendon relative to muscle force. The values for each muscle are identified by unique symbols.

around $30 F_0/L_c$ at forces greater than $0.3 F_0$. Estimates of stiffness were consistent between trials as displayed by the small size of the SD bars in Figure 7 (vertical lines). Even though specific stiffness is normalized to F_0 and L_c , there was considerable variability in the mechanical properties between specimens; maximal values ranged from 25 to $40 F_0/L_c$.

The specific stiffness of the distal portion of the aponeurosis was similar to that of the tendon (Fig. 8). Specific stiffness increased with muscle force up to $0.4 F_0$, whereas at higher force, its magnitude remained constant. The elastic properties were unaffected by the length of the fascicles except at $0.1 F_0$, where stiffness tended to increase with fascicle length.

Figure 9 displays the specific stiffness of the aponeurosis plotted against fascicle length at three different levels of force, 0.1, 0.2 and $0.6 F_0$, estimated from all muscles studied. Values based on recordings that include the proximal portion of the aponeurosis are also

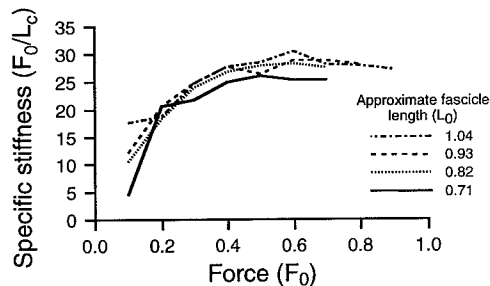


Fig. 8. *Felis catus*. The specific stiffness of the distal portion of the aponeurosis for contractions at four different muscle lengths. Stiffness increases with force up to $0.4 F_0$ and then remains constant at higher force levels.

displayed, but with corrections for the epimysial movement (see Appendix). The stiffness of the aponeurosis consistently increased with fascicle length at low force levels. At $0.1 F_0$, specific stiffness increased from 5 to $15 F_0/L_c$ as fascicle length increased from 0.8 to $1.1 L_0$. At $0.2 F_0$, the stiffness of the aponeurosis became less dependent on fascicle length: specific stiffness averaged around $15 F_0/L_c$, but ranged from 7 to $30 F_0/L_c$. At $0.6 F_0$, the specific stiffness of the aponeurosis averaged 25 to $30 F_0/L_c$. As in the estimates of the specific stiffness of tendon, there was considerable variability in the elastic properties of the aponeurosis between specimens even though stiffness was normalized to F_0 and L_c . However, note that there are no systematic variations in the specific stiffness estimated for different portions of the aponeurosis.

Figure 10A displays the relationship between tendon and aponeurosis stiffness in units of N/L_c at $0.6 F_0$ of muscle force and at a fascicle length of $1.0 L_0$. The stiffness of the entire aponeurosis, S_a , was strongly correlated with the stiffness of the tendon, S_t ($r = 0.96$; $P < 0.01$). The correlation was not different from unity ($P > 0.20$) so that the stiffness of the aponeurosis and tendon appear to be similar at least under moderate-to-high physiological loads. Figure 10B demonstrates the relationship between muscle force and the stiffness of the aponeurosis and tendon. Peak muscle force was found to be correlated with both the stiffness of the aponeurosis ($r = 0.94$; $P < 0.02$) and tendon ($r = 0.88$; $P < 0.05$). Therefore, the stiffness of the connective tissue was matched closely to the force capabilities of the muscle presumably by modifying the amount of connective tissue rather than its specific material properties. Surprisingly, when the stiffness estimates

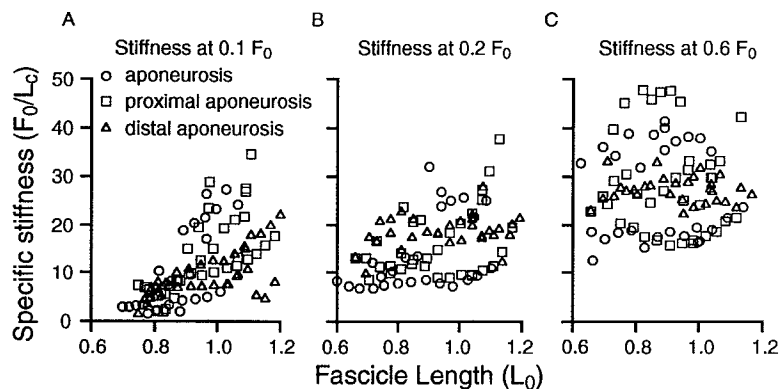


Fig. 9. *Felis catus*. The specific stiffness of the aponeurosis relative to fascicle length at three levels of muscle force, 0.1, 0.2, and 0.6 F_0 , estimated from all muscles studied. Circles denote estimates based on measurements of the entire aponeurosis. Squares and triangle

denote estimates based on measurements of the proximal and distal portions of the aponeurosis, respectively. Stiffness of the aponeurosis varies with fascicle length at 0.1 F_0 .

were normalized for peak muscle force there was still a significant positive correlation between muscle force and the specific stiffness of the aponeurosis ($r = 0.89$; $P < 0.05$). In other words, the stiffness of the aponeurosis normalized to muscle force was greater for stronger muscles and suggests that the elastic properties of the aponeurosis overcompensated for the force-generating capacity of these stronger muscles. This would also explain the large variability in the estimates of specific stiffness in Figure 9. There was a similar trend between the specific stiffness of the tendon and maximal muscle force, but this was not significantly different from zero ($P > 0.10$).

DISCUSSION

Overlying epimysium alters the recorded length of the aponeurosis

It has been claimed that the recorded length of the aponeurotic sheet in rat gastrocnemius and extensor digitorum longus muscles depended not only on muscle force, but also on muscle length (Huijing and Ettema, '88/89; Ettema and Huijing, '89). Our data demonstrate a similar interaction between fascicle length and the recorded length of the aponeurosis but show further that this effect was isolated to the most proximal end of the aponeurosis. Concurrently, Zuurbier et al. ('94) have also shown similar results for the

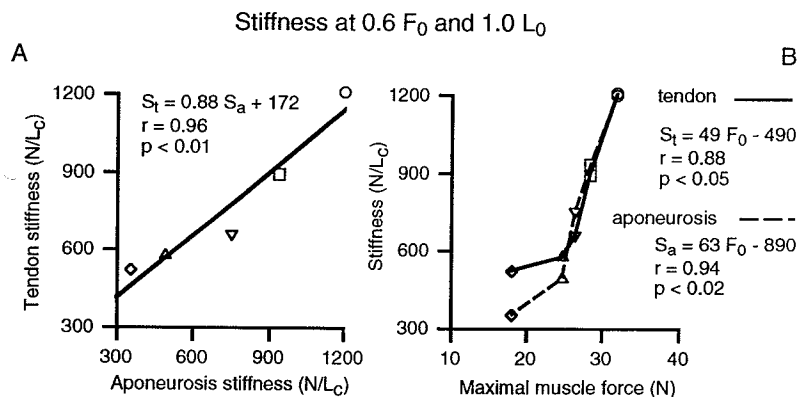


Fig. 10. *Felis catus*. **A:** Relationship between tendon and aponeurosis stiffness for each muscle. Symbols for each muscle are the same as in Figure 5. **B:** Relationships between tendon (solid) and aponeurosis (dashed) stiffness and maximal active force, F_0 , for each muscle.

aponeurosis of the gastrocnemius medialis (GM) muscle of Wistar rats. However, our observations of the aponeurosis surface revealed an overlying layer of epimysium along its perimeter that changed shape and slid over the aponeurosis when fascicle length changed. We propose that this epimysial movement may be responsible for the previously hypothesized interdependence between aponeurosis and fascicle length. This hypothesis appears to conflict with the study by Zuurbier et al. in which they removed epimysial tissue from the aponeurosis. However, they did not verify that the crossed-ply portion of the epimysium had been removed (C.J. Zuurbier, personal communication). Therefore, future studies must verify whether epimysial collagen is responsible for the apparent length changes in the aponeurosis.

Estimates of the stiffness of aponeurosis could be adversely affected by movement of the epimysium. Any technique that does not compensate for epimysial movement will incorrectly estimate the stiffness of tissue. To illustrate this point, the stiffness of the aponeurosis was estimated from records where one piezoelectric crystal was attached to the overlying epimysium (Fig. 11). The triangles in Figure 11B show the stiffness of the aponeurosis based on its length during a single contraction (dashed line in Fig. 11A) corrected for the movement of the epimysium. The circles denote the stiffness estimated from the same single contraction, but without correcting for epimysial movement. Epimysial movement during a single contraction appears to have caused the stiffness to be

overestimated by up to 5%. Alternatively, stiffness measures have been based on the length of aponeurosis when muscle was lengthened passively (Lieber et al., '91). Estimates of the stiffness of aponeurosis were less than 1 N/mm at low force levels. The low stiffness values were a consequence of the relatively flat relationship between passive muscle force and fascicle length. As a result, the lengths used to estimate stiffness spanned large changes in fascicle length that resulted in large epimysial movement and thus artifactual length changes in the recorded length of the aponeurosis. When the passive force-length curve increases rapidly at longer lengths, the stiffness levels rise. The stiffness of the aponeurosis could also be based on its length at the peak of isometric contractions recorded at different muscle lengths. This approach estimated that aponeurosis stiffness was around 5 N/mm at 15 N of force, much lower than estimates from length changes during a single contraction.

Not only did the epimysial movement cause artifactual measurement of movement of the aponeurotic sheet, but it also reduced the recorded velocity of the fascicles (Scott and Loeb, personal observation). In the present study, the crystal at the most proximal end of the aponeurosis was used to define the distal end of the fascicle. The observed movement of the epimysium covering the aponeurosis suggests that fascicle velocity would be underestimated by the ultrasonic technique. The percentage error can be computed from the slope of the linear regressions relating fascicle and aponeurosis length. These slopes

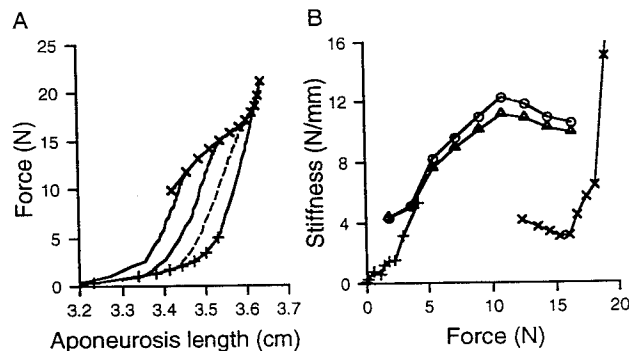


Fig. 11. *Felis catus*. **A:** The relationship between aponeurosis length and muscle force is complex when length measures include epimysial movement: +, passive force-length relationship; X, active force-length relationship; solid and dashed lines, force-length relationship during contractions at four different lengths. **B:** Stiffness

estimates of the aponeurosis are dependent on the type of force-length relationship used. +, X, same as in A; circles, stiffness estimates based on dashed line in A, but with no adjustments for changes in fascicle length; triangles, estimates based on dashed line adjusted for the effects from the overlying epimysium (see text).

range from 0.05 to 0.10, suggesting that approximately 5–10% of fascicle movement would be recorded as movement within the aponeurosis. Therefore, fascicle velocity would be 5–10% greater than recorded.

Width changes of the aponeurosis depend on fascicle length

The observed width of the aponeurosis increased when the fascicles shortened. The increase in aponeurosis width was most likely larger than recorded using the piezoelectric crystals because the crossed-ply network of collagen within the epimysium would have tended to decrease, not increase the width of the aponeurosis when the fascicles shortened. The long, narrow dimensions of the aponeurotic sheet of cat soleus make it difficult to interpret quantitatively estimates of aponeurosis width. Therefore, the sign, but not the magnitude, of the width changes can be interpreted from the present study. A more comprehensive recording of the aponeurosis using the piezoelectric crystals would be best suited on a broader, larger muscle such as the lateral or medial gastrocnemii of the cat.

The increase in the width of the aponeurosis when the fascicles shortened was probably related to the increase in cross-sectional area of the fibers beneath the aponeurosis. The volume of a muscle fiber remains constant during changes in its length. When the fiber shortens, the cross-sectional area of the fiber must increase (Elliott et al., '63; Trotter, '91). The observed increases in the width of the aponeurosis suggest that the fibers expand laterally to help compensate for their increased cross-sectional area. The diameter of the fiber in the sagittal plane probably also increases, even though no change in the length of the aponeurosis was observed. The longitudinal projection of the muscle fiber on the surface of the aponeurosis is proportional to the diameter of the fiber in the longitudinal plane and inversely related to the angle between the long axis of the fiber and the surface of the aponeurosis. The length of the aponeurosis remains constant when the muscle shortens, but the angle between the muscle fibers and the aponeurosis pennation angle is known to increase (Muhl, '82). Therefore, the diameter of the fiber in the longitudinal plane must increase. Presumably the diameter of the fiber increases in all directions simultaneously, with perhaps a small change in its cross-sectional shape (Trotter, '91).

The specific stiffness of aponeurosis is similar to tendon

The length-tension relation for tendon can be divided into two regions: a compliant toe region at low forces where tissue stiffness increases with force, and a linear region at higher force levels where stiffness remains constant (Ker, '81; Proske and Morgan, '87). This relationship would be expected from tendon given the crimped organization of its constituent collagen fibers (Diamont et al., '72). The nonlinear phase of the length-tension relation is associated with straightening out the collagen crimps whereas the linear phase is associated with extension of the collagen fibrils (Diamont et al., '72).

We observed that the organization of the collagen in the aponeurosis was similar to its organization in the tendon, except that it was spread across the surface of the muscle in longitudinal bands. The aponeurosis had a gradient of thickness from its proximal edge to its junction with the tendon that appears to match the accumulation of active tension that could be imposed on it by inserting muscle fibers. This would account for the similar strains that we observed in proximal and distal sections of aponeurosis. A more quantitative analysis of aponeurotic morphometry would require correction for the tapered and irregular cross section produced by its banded structure and measurement of the cross-sectional area of muscle fibers terminating at various levels. Moreover, the specific stiffness of the aponeurosis was similar to tendon, at least at moderate to high loads. This suggests that the specific stiffness along the entire length of the aponeurosis is constant and similar to that of the tendon.

The present estimates of the mechanical properties of the in-series connective tissue of cat soleus are similar to the results from previous studies (Walsmley and Proske, '81; Proske and Morgan, '84; Purslow, '89). Measures of stiffness in-series to the soleus fascicles by Rack and Westbury ('84) demonstrated a gradual increase in stiffness with force; assuming F_0 equalled 24 N, the specific stiffness rose to around $30 F_0/L_c$ at $0.3 F_0$. At higher force levels, Proske and Morgan ('84) recorded the stiffness of aponeurosis and tendon based on quick releases. Their estimate of tendon and aponeurosis compliance was 0.09 mm/N, which would also convert to around $30 F_0/L_c$. These values are quite similar to our results based on direct measures of the length of the aponeurosis and tendon

using ultrasound techniques. The advantage of the present technique is that stiffness estimates were possible for all levels of muscle force.

The mechanical properties of tendon have been extensively studied in many muscles and species (e.g., Abrahams, '67; Ker, '81; Lieber et al., '91). Assuming a cross-sectional area of 1.8 mm^2 (Rack and Westbury, '84), the Young's modulus of the soleus tendon in the present study equaled 420 N/mm^2 at higher loads. This is within the range of values recorded by other studies. Lieber et al. ('91) found values as low as 200 N/mm^2 for frog semitendinosus tendon, whereas Ker ('81) found stiffness estimates as high as $1,600 \text{ N/mm}^2$ for the plantaris tendon of sheep. The variability between measurements from different muscles and different species is probably related to differences in the actual properties of the connective tissue (Elliott and Crawford, '65). The apparent over-design of the aponeurosis and perhaps tendons of the largest muscles (Figs. 7, 10) may reflect such differences arising from sexual dimorphism; cats S9 and S10 that dominate this relationship were both large males.

Ker ('81) suggested that energy storage in tendon was largely due to the latter, linear phase associated with extension of the collagen fibers. This statement was based upon recordings of the length-tension relationship of plantaris tendon of sheep where the transition between the two phases occurred at low force levels (probably less than $0.1 F_0$). In contrast, Lieber et al. ('91) found that the length-tension relationship of tendon was never linear and its stiffness continuously increased up to F_0 . Rack and Westbury ('84) found that stiffness continued to increase with force for loads up to around $0.4 F_0$ for a portion of soleus tendon. In the present study, stiffness increased with force for loads less than $0.4 F_0$ and remained constant at greater force levels. The large variability between the transition between the two phases of tissue stretch relative to the force generating capacity of each muscle suggests that energy storage in the tendons of some muscles or species may be attributable to straightening of the crimped collagen as well as to extension of the collagen. Moreover, these differences may be related to functional differences between muscles. For example, the robust properties of the aponeurosis and tendon of the soleus muscle of the cat may be related to its normal

eccentric function during locomotion, which may produce forces greater than F_0 .

The stiffness of the aponeurosis (but not its length) appears to vary with fascicle length. Previous studies have suggested that there is no change in the stiffness of the entire series elastic component of muscle at different muscle lengths (Morgan, '77; Ettema and Huijing, '89). However, these studies concentrated on high force levels, where our results also showed no effect.

The variability in the stiffness of the aponeurosis at low force levels is probably related to its interaction with the terminating muscle fibers. It has been suggested that the stiffness of the myotendinous junction itself may change with fascicle length (Proske and Morgan, '87). However, the very small size of this structure in comparison to the length of the entire aponeurosis (microns compared to centimetres) suggests that the junction would have negligible effects on the total stiffness of the aponeurosis. It is more likely that the change in stiffness was related to the change in the shape of the aponeurosis when the muscle shortens. The crimped collagen in the aponeurosis is organized into longitudinal bands. The width of the aponeurosis increases when the muscle shortens. A portion of this lateral expansion may increase the width of the longitudinal collagen bands and perhaps increase the crimp of the collagen. An increase in the crimp of the collagen at shorter fascicle lengths would increase the range of the exponential spring behaviour, decreasing the apparent stiffness of the aponeurosis. At longer muscle lengths, the crimp angle would be reduced and thus the stiffness of the aponeurosis would rise more rapidly with applied force and would increase overall.

It is unlikely that the thin profile and sparse organization of the collagen network in epimysium could transmit any significant tensile load between muscle fascicles and adjoining aponeurosis. More likely, the major role of epimysium is to permit tendons and fascial planes to slide with respect to each other, acting as a lubricated layer that prevents adhesions from forming between such layers. Could there be a mechanical role for epimysium? Recently developed mathematical models have suggested that certain muscle architectures are unstable if superficial muscle fibers attach at an angle to the aponeurotic sheet (van Leeuwen and Spoor, '92). However, such theoretical models have not consid-

ered the potential stabilizing role of epimysium. It is possible that the mesh-like collagen network in epimysium is involved in stabilizing the anatomical organization of the underlying muscle fascicles and aponeurosis. As a consequence, an architectural model of muscle would be mechanically stable only if the overlying epimysial tissue were explicitly included in the model.

ACKNOWLEDGMENTS

We appreciate the helpful comments of Dr. F.J.R. Richmond and the technical assistance of J. Creasy, C. Simbirski and M. Yeoh. This work was supported by the Medical Research Council of Canada.

APPENDIX

Removal of the effects from the overlying epimysium on records of the aponeurosis

The contributions of the overlying epimysium to the recorded length of the aponeurosis can be estimated and corrected for. This correction is based on the assumption that the apparent shift in the aponeurosis length related to changes in the length of the fascicles is due entirely to epimysial sliding. To begin, the fascicles shortened slightly during the isometric contractions; the corresponding epimysial movement diminished the stretch imposed on the aponeurosis as muscle force increased. To remove the effect of epimysial movement on measures of aponeurosis length, a linear regression that related the length of the fascicle and of the aponeurosis was estimated at a muscle force equal to 0.5 F_0 , one-half the maximal isometric force generated by the muscle (McCall, '86). The slopes of these lines were always significantly different from zero ($P < 0.001$). The linear regressions were used to adjust the recorded length of the aponeurosis due to changes in fascicle length during each contraction.

The regression equation removed the effect of epimysial slip over the aponeurosis, but it did not remove the absolute length of epimysium recorded in the measures of aponeurosis length. The epimysium is not in series with the muscle fibers and thus would not stretch when force was transmitted by the aponeurosis. Therefore, aponeurosis stretch would be underestimated because a portion of the recorded length did not contribute to the length change. The approximate length of epimysium recorded along with the aponeurosis can be calculated by the ratio between epimysial and fascicle movement.

The shift in the aponeurosis length equals the change in length of the epimysium, ΔL_e . Since ΔL_e is proportional to the length change of the fascicles, ΔL_f , the length of the epimysium can be calculated using

$$\Delta L_f/L_0 = \Delta L_e/L_e \quad (1)$$

where L_0 and L_e are the lengths of the fascicle and epimysium at optimal length, respectively. Subtraction of L_e from the recorded length of the aponeurosis provides an estimate of the actual length of aponeurosis recorded by the piezoelectric crystals. These two procedures reduced the stiffness estimates by a total of 5–10%, compared to unadjusted values.

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