Relationship Between Achilles Tendon Mechanical Properties and Gastrocnemius Muscle Function

Strain was measured along the length of frog (Rana pipiens) gastrocnemius muscle-tendon units (MTU). Maximum muscle tension ($P_0$) was measured, and the MTU was passively loaded to $P_0$. Strain at $P_0$ was measured at eight intervals along the tendon and aponeurosis and was approximately two percent for all regions except the aponeurosis region closest to the muscle fibers where it was about six percent. A computer model predicted sarcomere shortening of up to 0.5 μm due to tendon lengthening which demonstrates that tendons provide a more complex physiological function than simply transmitting muscle force to bones.

Introduction

Movement represents the culmination of neural, muscular, and skeletal system interaction. Although each system has distinct properties, they do not act in isolation. Recent studies of tendon properties suggest that their role is much more complex than simple transmission of muscle force to bones. For example, tendons can function as energy stores (Cavagna, 1964; Alexander and Vernon, 1975; Morgan et al., 1978; Thompson et al., 1980), mechanical dampers (Hoffer et al., 1989; Griffiths, 1991), and force stabilizers (Rack and Ross, 1984; Rack and Westbury, 1984). Specifically, Alexander and Vernon (1975) demonstrated that, as kangaroos land, elastic components of the hindlimb and feet are stretched. This stretch is stored as elastic strain energy until take off when potential energy is converted into kinetic energy. Such energy storage greatly reduces the energetic requirements of locomotion. Similarly, Hoffer et al. (1989) and Griffiths (1991) demonstrated that the cat medial gastrocnemius (MG) tendon could act as a length absorber during gait which prevented excessive MG muscle stretch at high loads. In fact, these authors provided experimental records showing muscle shortening during tendon lengthening indicating that it is not appropriate to consider either muscle or tendon properties in isolation when trying to understand their physiological roles.

Few biomechanical tendon studies have been performed under physiological loading conditions. Those that have, provide a wide range of results: tendon stress at maximum muscle force ($P_0$) reportedly ranges from 3 to 80 MPa and tendon strain at $P_0$ reportedly ranges from 1.5 to 1.1 percent (Butler et al., 1978; Zajac, 1989; Lieber et al., 1991).

We recently measured frog semitendinosus (ST) tendon mechanical properties during passive loading to the tension level generated by the ST (Lieber et al., 1991). We found that the tendon and aponeurosis strained to different extents with the aponeurosis being more compliant. We then developed a model to simulate a fixed-end muscle contraction and found that sarcomere shortening at the expense of tendon lengthening distorted the sarcomere length-tension relationship (Lieber et al., 1992a). In other words, isometric force predictions based solely on muscle properties differed considerably with those predicted using both muscle and tendon properties. It is difficult to interpret the ST data in light of other studies in the literature for a number of reasons. First, most studies of muscle-tendon interaction have been performed on larger extensor muscles such as the gastrocnemius or quadriceps. It is conceivable that the design strategy of the ST may differ considerably since it is not an extensor muscle, and may not experience the higher forces normally supported by extensor muscles. Second, the ST has a relatively short tendon (about 2 mm) and aponeurosis (about 5 mm) which makes it technically difficult to compare the properties of the two regions since only one measurement can be obtained from each region, even with magnification. These limitations are not present in the gastrocnemius (GAST) the major frog ankle extensor which has a very long tendon (10 mm) and aponeurosis (20 mm).

Thus, the purposes of this study were to determine the mechanical properties of the frog achilles tendon and aponeurosis at loads similar to those generated by the GAST muscle and to predict the influence of achilles tendon mechanical properties on GAST muscle function.

Methods

Gastrocnemius Muscle-Tendon Preparation. Nine grass frogs (Rana pipiens) approximately six centimeters long were sacrificed by double pithing. The spine was transected at the most proximal portion of the ilium and the skin was removed from the leg. All dissection took place on a chilled glass plate and care was taken to ensure that the specimen remained wet to maintain muscle viability and osmotic balance. Ringer's solution was composed of (in mM): 115 NaCl, 2.5 KCl, 2.15...
Na₃H₂PO₄, 1.8 CaCl₂ and 0.85 NaH₂PO₄, adjusted to pH 7.0. The GAST was isolated by removing all hindlimb muscle except for its lateral and medial aspects, and the femoral origin attaching tendons, and the achilles tendon. The femur and the tibia were cut approximately 1 centimeter proximal and distal to the knee respectively. The achilles tendon was cut about one centimeter distal to the thickened tendon which traversed posterior to the heel, just proximal to the insertion on the plantar surface of the foot. The tibial nerve was isolated and transected as far proximally as possible.

The muscle-tendon unit (MTU) was patted dry and dye lines of elastin stain were applied to mark nine regions along its length (see lower panel of Fig. 2): thick tendon (the thickened tendon at the heel region), tendon, muscle fibers and six evenly spaced aponeurosis regions. The MTU was placed in a bath of chilled Ringer’s (14 ± 1 °C) circulating at about 40 ml/min. The knee joint was clamped to the arm of a servomotor (Cambridge Technology Model 310, Cambridge, MA) and the achilles tendon secured distal to the thickened tendon using a stationary clamp. The tibial nerve was placed in a suction electrode along with Ringer’s solution and a bipolar signal was delivered (Grass, model S88, Quincy, MA) through a stimulus isolation unit (Grass Model SIUS5B, Quincy, MA) for muscle activation. Stimulation and data acquisition were controlled using the computer system described by Lieber et al. (1986).

**Active Muscle Properties.** The activation threshold of each muscle was determined using single twitches (average value = 0.38 ± 0.06 V) and stimulation intensity was increased until no further increase in tension was noted (usually about 1 V). Testing voltage was set to approximately five times this voltage, guaranteeing supramaximal muscle activation. Muscle length was adjusted to the length at which muscle tension was maximal (L₀) using double-pulses of 0.3 ms duration separated by 22 ms. (Double-pulses were used instead of single-pulses to compensate for the relatively slack length of the muscle at L₀; Bahler et al., 1968). Three one-second stimulus trains were delivered at 100 Hz and 0.3 ms duration to determine muscle maximum tetanic tension (P₀).

**Passive Muscle-Tendon Mechanical Properties.** The MTU, set to L₀, was videotaped during cyclic loading from 0 to 100 percent P₀ at a strain-rate of approximately 0.1 percent per second as previously described (Lieber et al., 1991). Following five passive loading cycles, the muscle was readjusted to L₀ and P₀ was remeasured. This loading range was chosen as representative of those loads normally experienced by the frog GAST. While no direct data are available, analogous data obtained during cat locomotion suggest that loads from 10 percent P₀ to 150 percent P₀ are within the physiological range (Gregor et al. 1988; Walmsley et al. 1978).

Sarcomere lengths (Lₐ) were measured using the laser diffraction method (Lieber et al., 1984). The GAST central tendon (Dunlap, 1960) precluded whole-muscle laser diffraction requiring that the muscle be dissected down to a muscle fiber bundle (approximately 100 fibers) to enable laser diffraction. Control experiments on the ST, from which whole-muscle diffraction patterns could be obtained, demonstrated that sarcomere length values from whole-muscles and dissected bundles were not significantly different (difference = 0.065 ± 0.036 μm, p > 0.2). This was approximately the same difference as sarcomere lengths measured from different regions of the same whole ST muscle. The fiber bundle was deformed over thirty seconds from L₀ to the length corresponding to that reached during passively loading to P₀.

**Muscle Architectural Properties.** Contralateral muscles, with attached tendons, were weighed, secured on a piece of cork at L₀, fixed in 10 percent buffered Formalin for approximately 24 hours, digested for one hour in 15 percent sulfuric acid and stored in phosphate buffer (Sacks and Roy, 1982) for architectural determination as previously described (Lieber et al., 1991). Muscle physiological cross-sectional area (Aₚ) was calculated for each muscle as:

\[
Aₚ = \frac{(\text{muscle mass})(\cos \theta)}{(Lₘ)(ρ)}
\]

where \(Lₘ\) is the normalized fiber length, \(ρ\) is muscle density (1.056 g/cm³; Mendez and Keys, 1960) and \(θ\) is pennation angle. Fixation decreased mass by 35 ± 4 percent therefore muscle mass used in \(Aₚ\) calculations was adjusted for this loss after trimming the tendons.

Tendons were embedded in paraffin, sectioned (6-8 μm sections) and stained with haematoxylin and eosin (H and E). Tendon cross-sectional areas were measured from stained sections using an image analysis system (Image 1, Universal Imaging Corporation, West Chester, Pennsylvania). For each tendon specimen, three measurements were made from each section at each of three locations along the tendon (nine measurements per tendon).

**Data Analysis.** Passive connective tissue length changes and sarcomere lengths were measured from recorded images using a video-dimension analyzer (VDA; Physiological Instruments in Medicine, San Diego, CA) as described by Woo et al. (1980). Strains were calculated as:

\[
ε = \frac{Lₐ - L₀}{L₀}
\]

where \(Lₐ\) is tendon length, and \(L₀\) represents the original length prior to loading. Because there were no significant differences between strains measured from any of the last four loading cycles, all data reported were obtained from the fifth loading cycle. Passive load-strain relationships were determined for each region (Fig. 1). Curve fitting for the passive load-strain

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![Fig. 1](image)

Fig. 1 (A) Load-time relationship for frog gastronemius-tendon unit from 0 to 100 percent P₀. (B) Strain-time relationship measured from corresponding videotape record. (C) Load-strain relationship obtained from (A) and (B) at corresponding time points. Filled circles represent loading while open circles represent unloading.
data was performed by fitting individual data sets to an equation of the form:

$$
e = a + b \log_{10}(P),$$

where $e$ is strain, $P$ is tendon load and $a$ and $b$ are constants. Because data were measured over the same load range, all data participated equally in weighting the least-squares best fit. Next, the individual curve fit equations for each region were mathematically averaged yielding the average for all specimens and rearranged by solving for $P$ in Eq. (3) and substituting the condition that, at $e = 0$, $P = 10^{-6}$ (to satisfy the condition of zero load at zero strain) which yielded:

$$P = 10^{(e/a - b)} - 10^{(-e/b)}$$

These mechanical properties were incorporated into the muscle-tendon model previously described (Lieber et al., 1992a) to predict the effects of tendon compliance on GAST sarcomere shortening. Assumptions were identical to those presented for the ST.

**Statistical Analysis.** Strain at $P_0$ was compared between the nine regions by one-way analysis of variance (SuperANOVA, Abacus Concepts, Berkeley, CA). Multiple paired comparisons between all regions were performed post-hoc using the Fisher's Protected Least Squared Differences test with a significance level ($\alpha$) of 0.05. Comparisons between two regions along the tendon length were made using the unpaired Student's $t$-test. Data are presented as mean ± SD unless otherwise specified.

**Results**

**Muscle Properties.** GAST muscle $P_0$ (7.12 ± 1.59 N), length (30.57 ± 2.50 mm) and $A_{max}$ (31.55 ± 15.60 mm$^2$), were similar to those reported by (Table 1; Putnam and Bennett, 1993; Barry, 1990). Fiber length (9.67 ± 1.19 mm) was approximately one-third the length of the entire muscle. Passive $L_0$ at $L_0$ determined after passively stretching the MTU and dissecting the muscle down to a fiber bundle ($L_0$) was 2.95 ± 0.31 μm. The average passive sarcomere length tension relationship was expressed by the equation:

$$P_e = 10^{(e/a - b)} - 10^{(-e/b)}$$

**Table 1 Muscle properties measured**

<table>
<thead>
<tr>
<th></th>
<th>Gastrocnemius</th>
<th>Semitendinosus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle length (mm)</td>
<td>30.57 ± 2.50</td>
<td>22.56 ± 1.66</td>
</tr>
<tr>
<td>Fiber length (mm)</td>
<td>9.67 ± 1.19</td>
<td>10.52 ± 1.42</td>
</tr>
<tr>
<td>Sarcomere number</td>
<td>4.397 ± 539</td>
<td>4.781 ± 647</td>
</tr>
<tr>
<td>Maximum isometric tension (N)</td>
<td>7.12 ± 1.59</td>
<td>3.368 ± 0.170</td>
</tr>
<tr>
<td>Physiological CSA (mm$^2$)</td>
<td>31.55 ± 15.60</td>
<td>3.35 ± 1.30</td>
</tr>
<tr>
<td>Maximum isometric stress (MPa)</td>
<td>0.250 ± 0.056</td>
<td>107 ± 59</td>
</tr>
<tr>
<td>Fiber pennation angle ($)</td>
<td>9.5 ± 2.6</td>
<td>negligible</td>
</tr>
<tr>
<td>Sarcomere length at $L_0$ (μm)</td>
<td>2.95 ± 0.3</td>
<td>2.43 ± 0.06</td>
</tr>
</tbody>
</table>

*Data represent mean ± SD for $n$ = 9 independent samples.

*Data from Lieber et al. (1991).

*Measured after cyclic deformation.

**Tendon Properties.** The aponeurosis was twice as long (21.40 ± 3.13 mm) as the tendon (10.34 ± 1.38 mm). Interestingly, in spite of large variations in cross-sectional area, width and gross morphology (Table 2), surface strain was remarkably consistent along the tendon-aponeurosis length. In fact, one-way ANOVA revealed no significant differences between any of the tendon-aponeurosis regions save the most proximal aponeurosis region (Fig. 2). Strain at $P_0$ in these regions ranged from 1.8 to 2.5 percent while strain in the most proximal aponeurosis region was over 6 percent.

Passive load-strain curves of the tendon region, thick tendon region and all six aponeurosis regions (a total of eight regions) were similar in form (Fig. 3) and their load-strain relationships could be explained by the equation:

$$\text{Load (}\%P_0) = 10^{(e/a - b)} - 10^{(-e/b)}$$

with coefficients of correlation ranging from 0.81 to 0.95. Since no significant differences in strain at $P_0$ were observed between tendon and distal aponeurosis regions, they were considered as a single functional unit (Fig. 4; $a = -0.2566$, $b = 1.2513$) while the most proximal aponeurosis region was considered separately ($a = -0.65262$, $b = 3.3209$).

Tendon cross-sectional area varied almost three-fold along its length (Table 2). Thus the thickened tendon in the heel region (2.83 ± 0.93 mm$^2$) was significantly greater in cross-sectional area than the rest of the tendon (0.98 ± 0.32 mm$^2$, $p < 0.001$). Consequently, maximum stress for the tendon region (9.39 ± 2.97 MPa) was over twice that observed for the thickened tendon region (3.78 ± 2.12 MPa) and its Young's modulus (1.548 ± 683 MPa) was greater than that of the thick tendon (632 ± 280 MPa).

**Table 2 Tendon properties**

<table>
<thead>
<tr>
<th></th>
<th>Gastrocnemius</th>
<th>Semitendinosus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tendon length (mm)</td>
<td>10.34 ± 1.38</td>
<td>4.95 ± 0.75</td>
</tr>
<tr>
<td>Aponeurosis length (mm)</td>
<td>21.40 ± 3.13</td>
<td>5.54 ± 1.06</td>
</tr>
<tr>
<td>Tendon CSA (mm$^2$)</td>
<td>0.980 ± 0.321</td>
<td>0.118 ± 0.040</td>
</tr>
<tr>
<td>Tendon stress at $P_0$ (MPa)</td>
<td>9.39 ± 2.97</td>
<td>3.08 ± 0.63</td>
</tr>
<tr>
<td>Tendon strain at $P_0$ (%)</td>
<td>2.42 ± 0.93</td>
<td>4.43 ± 2.82</td>
</tr>
<tr>
<td>Aponeurosis strain at $P_0$ (%)</td>
<td>2.90 ± 0.91</td>
<td>7.95 ± 4.74</td>
</tr>
<tr>
<td>Tendon length/fiber length ratio</td>
<td>3.39 ± 0.43</td>
<td>1.01 ± 0.19</td>
</tr>
<tr>
<td>Tendon/muscle CSA ratio</td>
<td>0.0358 ± 0.0142</td>
<td>0.0362 ± 0.0130</td>
</tr>
<tr>
<td>Tendon Young's modulus at $P_0$ (MPa)</td>
<td>1.548 ± 683</td>
<td>188 ± 21</td>
</tr>
</tbody>
</table>

*Data represent mean ± SD for $n$ = 9 independent samples.

*Data from Lieber et al. (1991).
Histological sections from along the tendon showed regional variations in staining intensity (Fig. 5). The darkest regions within a section were organized into what appeared to be the typical collagenous matrix while the lightest regions (observed in the thick tendon) contained randomly-oriented collagen fibers, enmeshed in a matrix of cells, which morphologically resembled hyaline cartilage.

**Computer Model Results.** Using measured tendon and aponeurosis mechanical properties, sarcomere length changes during fixed-end contractions were calculated for initial sarcomere lengths ranging from 1.3 to 3.7 μm (Fig. 6A). For example, sarcomeres initially at 2.7 μm shortened 0.5 μm to 2.21 μm upon activation and thus produced more tension than would be predicted based solely on their initial length. In this case, these sarcomeres increased their ability to generate tension from 70 percent $P_0$ to 100 percent $P_0$ (Fig. 6B). The envelope of sarcomere length-tension records (Fig. 6B) is the classic sarcomere length-tension curve (Gordon et al., 1966).

A sarcomere placed in series with a frog achilles tendon and aponeurosis would thus shorten upon activation to produce forces different than those predicted without including tendon compliance. The effect of this shortening would be a skewing of the peak of the sarcomere length-tension curve to higher lengths by about 20 percent. In addition, tendon compliance also shifted the peak of the MTU length-tension curve by 2.2 mm, or about five percent and the MTU operating range increased by 0.8 mm (two percent).

**Discussion**

The main result of this study was that strain is relatively constant along the length of the frog achilles tendon at loads equal to GAST $P_0$. Additionally, this strain of approximately two percent distributed along the tendon-aponeurotic complex permits significant sarcomere shortening to occur during fixed-end contractions.

The current results present a contrast to our previous study of the frog ST in which tendon compliance was significantly less than aponeurosis compliance. However, in both cases the majority of the connective tissue strained about two percent at $P_0$. This was especially interesting in the GAST where connective tissue cross-sectional area varied three-fold along its length and even appeared to have morphological material variations. Conceivably, tendons are able to regulate mechanical properties along their length independent of actual composition in order to maintain a relatively constant strain. In this sense, the connective tissue system could behave like bone, which some have suggested regulates trabecular dimensions and density to maintain constant strain despite geometric variation. This concept is supported by the observation that the ratio of muscle/tendon CSA was nearly identical for the GAST and ST systems (Table 2).

In spite of the observation of similar material properties,
![Graphs showing sarcomere length-time and tension-length relationships.](image)

**Fig. 6** (A) Sarcomere length-time records predicted during muscle activation. Open circles represent the case for a specific initial sarcomere length of 2.70 μm which shortens 0.5 μm to 2.21 μm after activation. (B) Sarcomere length-tension relationships predicted during muscle activation. Open circles represent an initial length of 2.70 μm which shortens upon activation and increases its ability to generate tension from 70 %P_0 to 100 percent P_0.

**Table 3** Muscle-tendon unit examples

<table>
<thead>
<tr>
<th></th>
<th>L_/L_f Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog gastrocnemius</td>
<td>3.30</td>
</tr>
<tr>
<td>Human vastus*</td>
<td>2.65</td>
</tr>
<tr>
<td>Human gastrocnemius*</td>
<td>8.85*</td>
</tr>
<tr>
<td>Human soleus*</td>
<td>11.25*</td>
</tr>
<tr>
<td>Human hamstrings</td>
<td>3.60</td>
</tr>
<tr>
<td>Human extensor digitorm communis</td>
<td>2.5*</td>
</tr>
<tr>
<td>Human extensor indicis proprius</td>
<td>31*</td>
</tr>
</tbody>
</table>

*Values from Hoy et al. (1990).

Values calculated from Lieber et al. (1992a) and Horii et al. (1992).

However, probably the most important parameter to compare between the two MTUs is the tendon length/fiber length ratio (L_/L_f) which was 3.30 ± 0.43 for the GAST and only 1.01 ± 0.19 for the ST (Table 2). In this context, “tendon length” refers to the total connective tissue length in series with muscle fibers, i.e., tendon + aponeurosis. Thus, the GAST would be considered a more compliant MTU than the ST.

When incorporating tendon compliance and dimensions into a structural model, we found that GAST sarcomeres shortened twice as much as ST sarcomeres (Fig. 7A). This resulted in a skew of the isometric sarcomere length-tension curve to higher lengths by about 20 percent, whereas the ST peak was shifted by only 10 percent. We checked the idea that differences between the two MTUs were due to tendon dimensions and not tendon material properties by using the ST model to predict sarcomere length changes at different L_/L_f ratios ranging from 1.0 to 10. Sarcomere length change increased with an increase in L_/L_f as expected, and there was very little difference between the ST model for L_/L_f of 3.3 (the value of the GAST MTU) and the actual data from the GAST model (Fig. 7B). Length-tension relationships corresponding to these conditions were also determined and again, there was little difference between the ST model prediction for a L_/L_f of 3.3 and that of the GAST. This supports the theory that L_/L_f is a parameter with great influence on muscle force generation.

Several other MTUs appear to have L_/L_f ratios which suit their function (Table 2). For example, the human plantar flexor tendons could be expected to act as energy stores during running. Such an effect has already been observed in the red kangaroo (Alexander and Vernon, 1975) and the cat (Hoffer et al., 1989; Griffiths, 1991). The extensor digitorum communis and extensor indicis proprius muscles also have a high L_/L_f ratio which could be considered adaptive for two reasons. First, increased tendon material serves to increase the functional range of the MTU (Zajac, 1989) and, in the fingers and thumb, the tendon can act to dampen force changes accompanying small movements, thus enhances force control of the fingers (Rack and Westbury, 1984; Rack and Ross, 1984).

On the other end of the spectrum, it might be hypothesized that the low L_/L_f ratio is appropriate for the frog ST. Previous studies suggested that the ST is actually actively extended during the hop, most likely by the knee extensors (Mai and Lieber, 1990). A long tendon in the ST could hinder this function. Finally, an exception to the rule regarding L_/L_f ratios was recently described for the kangaroo rat achilles tendon, which had a much higher relative stiffness than the full-sized kangaroo. It was hypothesized in this case that this high tendon stiffness was selective for rapid acceleration to avoid predation at the expense of energy storage (Blewener and Blickhan, 1988).

**Fig. 7** (A) Sarcomere length change predicted as a function of initial sarcomere length from the frog gastrocnemius (closed circles, present study) and the semitendinosus (open circles, data from Lieber et al., 1991). (B) Sarcomere length change predicted as a function of initial sarcomere length from the semitendinosus model for muscle-tendon units with tendon length/fiber length ratios 1, 3.3, 5, and 10 (open circles). Closed circles represent data from the gastrocnemius curve in (A). This simulation suggests that it is the amount of connective tissue which primarily determines its influence on skeletal muscle.

The influence of tendon compliance on the GAST and ST were quite different. While the GAST and ST have similar muscle and fiber lengths (Table 1), the GAST muscle cross-sectional area is 10 times that of the ST and its P_0 is 20 times greater.
Acknowledgments

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References


