THE PRODUCTION OF MOVEMENT results from coordinated action between nervous, muscular, tendinous, and skeletal systems. Numerous studies have elucidated properties of the individual component systems and have then postulated their roles during normal movement. We previously attempted to integrate muscular and skeletal properties by measuring frog semitendinosus (ST) muscle sarcomere length (L_s), joint kinematics, and joint torque in vitro (11). We then used these data to predict the time course and nature of ST muscle properties during a simulated hop (13).

As a first approximation, our studies neglected the influence of tendon compliance, a property which has recently received a great deal of attention. For example, Hoffer et al. (7) demonstrated simultaneous tendon lengthening and muscle fiber shortening in the cat medial gastrocnemius and Achilles tendon during locomotion. As a result, they suggested that in situ muscle fiber length was not the same as muscle-tendon length observed during locomotion. Zajac (25) reviewed the various factors that contribute to the properties of the muscle-tendon unit and demonstrated that failure to include tendon compliance in measurements of muscle properties leads to erroneous conclusions.

In light of these reports, and our desire to understand the interrelationship between muscular, tendinous, and skeletal systems, we measured the properties of the ST-tendon-bone complex. Although numerous authors have measured tendon material and structural properties (for review, see Ref. 4), few studies have integrated muscle and tendon properties (9, 15–17, 22), and almost all of these studies have concentrated on the Achilles tendon or other long tendons associated with large mammalian extensor muscles. The frog ST offers the advantage of permitting noninvasive L_s measurement during measurement of tendon mechanical properties and is the only muscle for which the sarcomere length-tension relationship has been elucidated (6). Thus the purpose of this investigation was to measure the mechanical properties of the frog ST tendon, bone-tendon junction, and muscle-tendon junction (aponeurosis) during passive loading to a tension equal to maximum tetanic contraction. A brief account of this work has been presented previously (12).

METHODS

Muscle-tendon preparation. The model chosen for this study was the muscle-tendon unit of the frog ST muscle (*Rana pipiens*). This model was chosen based on the muscle’s well-established sarcomere length-tension properties (6) and previous studies establishing the relationship between muscle and joint properties (11, 13). Frogs were killed by double pithing (n = 14 independent experiments), and the ST-tendon unit was carefully isolated along with its attachments to the pelvis and tibia. The bones of the bone-muscle-tendon (BMT) unit were clamped to specially designed fixtures that permitted viewing of the bone-tendon interface while maintaining secure contact with the BMT unit (Fig. 1, inset). The BMT unit was submerged in chilled (17 ± 2°C) Ringer solution composed of (in mM): 115 NaCl, 2.5 KCl, 2.15 Na_2HPO_4, 1.8 CaCl_2, and 0.85 NaH_2PO_4, adjusted to pH 7.0. One clamp was fixed to the moving arm of a dual-motor model 310 (Watertown, MA). Dye lines (elastin stain) were applied at intervals along the BMT unit partitioning it into three regions (Fig. 1; inset): a region containing the bone-tendon interface (referred to as the bone-tendon junction), a region containing only the bare
tendon (tendon), and a region containing the muscle-tendon junction (aponeurosis). Boundaries between these regions were defined somewhat arbitrarily based on morphological appearance. This might account for some of the variability within regions between samples. The lengths of these regions were, nominally, 3, 2, and 6 mm, respectively. The experiment was videotaped using a 70 mm, 1:3.5 macro lens and super VHS videotape recorder (Fig. 1).

Before measurement of tendon tensile properties, muscle stimulation threshold was determined (in mV) and stimulation intensity was increased until no further increase in tension was observed. Activations were then performed at 10 times this intensity (150-V pulses yielding a field strength of ~50 V/mm). Stimulation configuration implemented cylindrical platinum electrodes closely approximating the muscle length to ensure that current passed through the muscle fibers and not merely around them via the Ringer solution. Maximum stimulator current output (250 mA) was sufficient to fully activate the muscle in the Ringer solution. Muscle length was then adjusted to the length at which tension was maximum when elicited by double pulses of 3-ms duration ($L_0$). Double pulses were used to compensate for the relatively “slack” muscle-tendon unit at these lengths. This occurred at a passive sarcomere length of ~2.5 μm as determined by optical laser diffraction. Next, maximum tetanic tension ($P_o$) was measured by directly activating the muscle for 1.5 s at 100 Hz, 0.3-ms pulse duration at the supramaximal intensity.

**Measurement of tendon mechanical properties.** Pilot experiments revealed that the traditional method of deforming the material and measuring load to determine mechanical properties was unsuitable for this system. We measured dramatic decreases in $P_o$ following constant deformation experiments which induced loads that often exceeded three times $P_o$, even when adjusting deformation magnitude to muscle fiber and tendon architecture. To avoid this problem, we conducted the experiments under conditions of controlled loading (Fig. 2). After adjusting muscle length to $L_0$, and measuring $P_o$ and $L_0$, BMT units were cyclically loaded to $P_o$ (5 cycles) in ~30 s (Fig. 2A, nominal tendon strain rate, 0.1%/s). This relatively low strain rate was chosen to permit comparison with tendon mechanical properties similarly obtained (4). The entire loading sequence was videotaped for later strain analysis. The BMT units were then reset to $L_0$, and the muscle was positioned so that a clear diffraction pattern could be obtained by transillumination using a HeNe laser (10). The BMT units were again cyclically loaded with five load-release cycles while videotaping the laser diffraction pattern. Finally, the muscle was reset to $L_0$ and activated to $P_o$ to check for preparation deterioration. The average postloading $P_o$ was 91 ± 8% (SD) of the preloading value, and thus fatigue/damage to the muscle contractile apparatus was considered negligible.

**Muscle architectural properties.** BMT units were removed from the apparatus, secured at $L_0$, and fixed in 10% buffered Formalin for determination of muscle architectural properties according to a modification of the methods of Sacks and Roy (19). ST muscle length was measured as the distance from the most proximal fiber origin to the most distal fiber insertion. Three single fibers (single fibers completely traversing from 1 tendon to the other) were dissected from the ST (1 each from the proximal, middle, and distal regions), and their fiber length ($L_f$) and $L_0$ were measured, enabling calculation of sarcomere number. The remaining BMT specimen was weighed and embedded in paraffin for routine processing, sectioning (8-μm sections), and histological staining with hematoxylin and eosin.

Muscle physiological cross-sectional area ($A_{cm}$) was calculated for each muscle (19) as

$$A_{cm} = \frac{(\text{muscle mass}) \cos \theta}{(L_f)(\rho)}$$

where $\theta$ was pennation angle (3°, Ref. 11) and $\rho$ was muscle density (1.056 g/cm³, Ref. 14). On the basis of the need to preserve tendon integrity for area measurements (see below), it was necessary to estimate muscle mass based on measurement of muscle plus tendon mass. Tendon mass for each specimen was measured from the contralateral leg and was subtracted from the total mass to yield muscle mass.

Tendon cross-sectional area was calculated by averaging the areas of three or four histological serial sections that were traced onto paper using a drawing tube attached to a light microscope. Normal tendon cross-sectional morphology (tightly packed tendon fascicles which were filled with collagen fibrils) was ensured before such measurements were made. Sections with obvious cracks or sectioning artifacts were excluded. Error introduced by repeated measures within a given section was 1.1%, while the error introduced by repeated measures of different sections within the same tendon was 4.3%. In pilot experiments, tendon shrinkage due to fixation was estimated as ~5% (by comparing fresh snap-frozen samples
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at −159°C with Formalin-fixed samples) and was ignored in area calculations. We were not able to make a reasonable estimate of aponeurosis or bone tendon junction cross-sectional area in light of the complex geometry of these regions, and, therefore, no stress-strain relationships were generated for them.

Strain at Po in different regions of the BMT complex during passive loading was measured using the video dimensional analyzer as described extensively by Woo et al. (23). Strain was measured from the fifth cycle of the cyclic loading (Fig. 2B). Pilot studies demonstrated no significant difference between peak strains measured from any of the final four cycles. All data reported (unless otherwise noted) refer to data obtained from the fifth loading cycle. With the use of corresponding points from the load-time relationship (Fig. 2A) and strain-time relationship (Fig. 2B), the load-strain curve was constructed (Fig. 2C).

Statistical analysis. Strains in the three different connective tissue regions were compared by one-way analysis of variance (ANOVA). Assumptions of ANOVA (i.e., normality and homoscedasticity) were explicitly tested using diagnostic software (20). In cases in which the data set failed the ANOVA assumptions, mathematical transformations were implemented. Multiple paired comparisons between the three regions were performed post hoc using Fisher’s protected least-squares difference method. Significance level (α) was chosen as 0.05. Because of the parameter’s relatively small standard deviation (σ) and the large sample size (n = 14), statistical power (1 − β) exceeded 90% for all parameters measured. Data are presented in the text as means ± SE unless otherwise noted.

Curve fitting for the passive load-strain data was performed by first transposing the data set and fitting individual data sets to a curve of the general form: ε = a + b log10 (%Po), where ε represents strain. In this way, because all data were measured over the same load range, all data participated equally in weighting the least-squares best fit. Correlation coefficients for individual data sets always exceeded 0.7, indicating good individual fits. Next, curve fit equations were mathematically averaged to yield the best fit for the overall data set and then transposed into the form: %Po = 10(ε−a)/b, for a given region.

RESULTS

Muscle properties. Muscle properties measured were similar to those previously reported by Lieber and Boakes (Table 1; Ref. 11). Maximum tetanic tension (0.4 N) and active stress (i.e., specific tension, 107 kPa) were slightly lower than the typical 200 kPa reported for frog skeletal muscle (6). However, given a muscle Q10 of ~2 for Po (5) and the fact that the experiments were performed at relatively high temperatures (~20°C), this value appears reasonable. In addition, our architectural calculations of A, would tend to be larger than those typically obtained by direct measurement, thus decreasing our value for specific tension. Tendon, bone-tendon junction, and aponeurosis length (2.1, 2.8, and 5.5 mm, respectively; Table 1) were relatively short compared with muscle fiber length (10.5 mm). Based on the staggered arrangement of muscle fibers in this preparation (where we could follow individual fibers from origin to insertion), we surmised that each muscle fiber was organized in series with one “length” of aponeurosis and two lengths each of tendon and bone-tendon junction (one at the pelvic and one at the tibial end). Thus the ratio of connective tissue length to muscle fiber length was 1.5 (calculated as [(2.8 × 2) + (2.1 × 2) + 5.5]/10.5), which would cause the ST system to be considered a relatively “stiff” muscle-tendon unit (cf. Ref. 25).

Passive Io at Io was 2.45 ± 0.06 μm (Table 1), which
is considerably greater than the single fiber active optimal sarcomere length at $P_0$ of 2.0–2.2 μm (6). This was probably partly due to the fact that $L_o$ was determined using double-twitch pulses (which results in a longer $L_o$ than that obtained using tetanic contraction; Ref. 2) and also to the fact that sarcomere shortening occurred during tension generation. At $L_o$, passive tension was negligible. In fact, cyclic loading of the BMT complex resulted in an initial “stretching” of the muscle-tendon unit such that upon unloading $L_o$ returned only to 3.44 ± 0.12 μm. In this experimental system, therefore, slack length was not identical to optimal length.

**Tendon mechanical properties.** Load-strain curves for all three regions, expressed in terms of $P_0$, failed to reveal the usual compliant toe region followed by a linearly increasing load. Rather, in all experiments, load increased exponentially with increasing strain (Fig. 3). A fair amount of variability between animals within a region was observed in spite of normalization of force to maximum tetanic tension and the use of strain, which is itself a normalized parameter. Our conclusion was that this variability resulted primarily from the arbitrary division of the tendon into bone-tendon junction (BTJ), tendon, and aponeurosis regions. In spite of this variability, several clear trends were observed. Generally, aponeurosis strain was greater than BTJ strain, which was greater than tendon strain (Fig. 3, A–C). At $P_o$, the strain in these three regions was 8.0, 3.4, and 2.0%, respectively, yielding a compliance ratio of 4.0:1.7:1.0 (Table 1). These strains were significantly different between all three regions ($P < 0.005$, 1-way ANOVA), with the aponeurosis strain being significantly more than the tendon or BTJ ($P < 0.05$). There was no significant difference between BTJ strain and tendon strain at $P_o$ ($P > 0.4$).

When data were normalized to strain at $P_o$ (i.e., strain at $P_o$ defined as 100%), there was no significant difference between regions with respect to the shape of the curve and much less variability observed within regions (Fig. 4). We provisionally interpret these data to mean that material properties of the various regions are similar, and measured strain differences are due to regional size variation.

$L_o$ during passive loading. $L_o$ measurement during passive loading provided a measure of muscle “strain” based directly on the relative sliding of actin and myosin filaments, not simply surface markers. Similar to the tendon results, load-$L_o$ curves were curvilinear, indicating changing passive muscle stiffness with load (Fig. 5). Interestingly, the load-$L_o$ curves were much less variable than the tendon load strain curves (compare Figs. 3 and 5).

Maximum tendon stress at $P_0$ (Fig. 6) was ~3 MPa, which was ~30 times greater than maximum muscle stress (~100 kPa) owing to the large muscle $A_o$ (3.5 mm² compared with 0.12 mm² for the tendon; Table 1). Be-
cause these stress strain data were well approximated either by an exponential curve or by a polynomial curve of degree 2 ($r^2 = 0.43; P < 0.001$; Fig. 4), the calculated Young's modulus was obviously not constant but linearly increased as a function of load. In the tendon region, Young's modulus ranged from 0.1 GPa at 1% tendon strain to 2 GPa at 4% strain. Tendon modulus at $P_o$ was 0.188 GPa (Table 1).

FIG. 4. Normalized load-strain data for all regions. Strain was normalized to the maximum strain at $P_o$, and load was normalized to maximum load at $P_o$. Note that the 3 regions demonstrate considerable overlap.

FIG. 5. Load-sarcomere length curves for all data. Note that load varied with sarcomere length ($L_s$) in a curvilinear fashion, suggesting that muscle stiffness was also not a constant. These data were well approximated by the relationship $\%P_o = 10^{L_s - 0.45} (-0.52; P < 0.05)$.

FIG. 6. Stress-strain relationship for frog ST tendon. Note that, at $P_o$, tendon stress was ~3 MPa and tendon strain was ~2%. The exponential equation of best fit for these data is stress (MPa) = $10^{1.10(1.10) - 1.2} (r^2 = 0.43, P < 0.05)$.

FIG. 8. Stress-strain relationship for frog ST tendon. Note that, at $P_o$, tendon stress was ~3 MPa and tendon strain was ~2%. The exponential equation of best fit for these data is stress (MPa) = $10^{1.10(1.10) - 1.2} (r^2 = 0.43, P < 0.05)$.

FIG. 9. Load-sarcomere length curves for all data. Note that load varied with sarcomere length ($L_s$) in a curvilinear fashion, suggesting that muscle stiffness was also not a constant. These data were well approximated by the relationship $\%P_o = 10^{L_s - 0.45} (-0.52; P < 0.05)$.

FIG. 10. Normalized load-strain data for all regions. Strain was normalized to the maximum strain at $P_o$, and load was normalized to maximum load at $P_o$. Note that the 3 regions demonstrate considerable overlap.

FIG. 11. Load-sarcomere length curves for all data. Note that load varied with sarcomere length ($L_s$) in a curvilinear fashion, suggesting that muscle stiffness was also not a constant. These data were well approximated by the relationship $\%P_o = 10^{L_s - 0.45} (-0.52; P < 0.05)$.

FIG. 12. Stress-strain relationship for frog ST tendon. Note that, at $P_o$, tendon stress was ~3 MPa and tendon strain was ~2%. The exponential equation of best fit for these data is stress (MPa) = $10^{1.10(1.10) - 1.2} (r^2 = 0.43, P < 0.05)$.

DISCUSSION

The purpose of this investigation was to elucidate tendon mechanical properties under loads and conditions that were considered "physiological." The best examples of in situ physiological loads have been obtained in cats during level locomotion. In these cases, Achilles tendon tension ranges from near zero to slightly greater than $P_o$ (21). Previous tendon studies using isolated material have led to the following generalized conclusions (which were recently summarized by Zajac (25)): 1) tendon stress-strain curves are composed of a compliant toe region followed by a linear region, 2) tendon modulus (the slope of the stress-strain curve) is approximately constant and is ~1.2 GPa, 3) tendon modulus is independent of strain rate at physiological strain rates, 4) tendons fail at a stress of ~100 MPa, 5) tendon and aponeurosis have similar mechanical and material properties, and 6) tendons strain from 2 to 10% during loads near $P_o$, at which point the stress is ~30 MPa. In the current study, we obtained data which do not support all of these assertions: tendon and aponeurosis mechanical properties differed from one another, tendon stiffness varied considerably as a function of load, and tendon load and stiffness at $P_o$ were much lower than expected. In support of these assertions, tendon strain ~2% at loads equivalent to $P_o$. It was not possible to measure failure properties.

Different properties of tendon vs. aponeurosis have been reported by Huijing and Ettema (9) for the rat medial gastrocnemius tendon and by Rack and Westbury (18) for the "bare" external tendon compared with the entire external and internal cat soleus tendon. However, other investigators have concluded that tendon and aponeurosis properties are similar (16). It should be noted that dramatic methodological differences as well as species differences may contribute to the general lack of consensus.

A surprising result of this study was the relatively low tendon stress and stiffness measured at $P_o$. (3 MPa compared with the expected 30 MPa, and 0.2 GPa compared with the expected 1.2 GPa). Inspection of previous reports reveals that most stiffness measurements were obtained from the "linear" portion of the stress-strain curve that occurs at relatively high stresses (≥20 MPa), whereas the stresses used in the current study were lower. The tendency for tendon stiffness to be low at low loads, such as those used in the current study, was demonstrated by Rack and Westbury (17) and Walmsley and Proske (22) in the cat soleus, which supports the concept that, under physiological loads, tendon properties may be quite different from those extrapolated from isolated tendon preparations. Because our methods of measuring tendon cross-sectional area (CSA) from fixed tendon sections would tend to underestimate CSA and thus overestimate stress, these low stresses are even more surprising. Caution must be observed, therefore, in using modulus values obtained from studies implementing widely varying methodologies.

Compliance and elastic strain energy storage are positively correlated (1). Thus it might be argued that the frog ST is actively involved in energy storage and release,
especially in light of the prediction that the muscle experiences stretch shorten cycles (13). However, this explanation is weak in light of the relatively short length of tendon in series with the ST (ratio = 1.5) compared with, say, the length of Achilles tendon in series with the gastrocnemius (ratio = 11; Ref. 8). Thus, although the material properties of the ST seem well suited for elastic strain energy storage, there does not appear to be enough material in series with the muscle fibers for this mechanism to be effective. In contrast to the current findings of increased compliance in a small animal, Biewener and Blickhan (3) recently showed increased stiffness in the kangaroo rat Achilles tendon (CSA = 1 mm²) compared with the kangaroo tendon (CSA = 15 mm²) and concluded that it was adaptive to avoid predation. It should be noted that the current study is one of the very few (however, cf. Ref. 9) in which the properties of a flexor and not an extensor were measured. In the same way that muscle properties differ dramatically between flexors and extensors, it may not be surprising that tendon properties might also differ significantly. In fact, Woo et al. (23) reported higher ultimate loads and stiffnesses in pig digital flexors compared with extensors.

At a force equivalent to Pₚ₀, tendon strain was 2.0% while aponeurosis strain was 8.0%. This tendon strain value is relatively close to that reported by Rack and Westbury (18) for the cat soleus during low force contractions. Because the upper limit of the toe region occurs at ~4% strain (4), this implies that the physiological operating range of this muscle-tendon unit is in the toe region.

In spite of the rough agreement on tendon strain magnitude (2% to 8%) during muscle contraction, it is difficult to compare directly the results from different studies. The main reason for this difficulty is the lack of agreement on a definition of 0% strain. In his review, Zajac (25) defined 0% as the slack length, the length at which passive tension was zero. However, in many studies of isolated tendons, a small passive preload was applied to the tendon before testing. This would have the effect of increasing the tendon stiffness and decreasing the measured (or predicted) strain. In fact, it is possible that this procedure actually brings the tendon outside of its physiological operating range.

In summary, our tendon measurements under physiological conditions suggest that the mechanical properties of different tendon regions vary considerably and that they operate almost exclusively in the toe region of the classic stress-strain curve. Clearly these results would have significant implications in motor control strategies, and they may allude to yet another level of complexity designed into the musculoskeletal system. Current motor control ideas point to the fact that the peripheral "effectors" are themselves extremely complex in design and permit more simple "control" signals to elicit the types of movements desired. Further studies on different muscle groups within the same animal are required to elucidate such general principles.

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