Sarcomere Length-Joint Angle Relationships of Seven Frog Hindlimb Muscles

Key Words
Skeletal muscle
Sarcomere length
Length-tension relationship
Joint torque
Sarcomereogenesis
Myofibrillogenesis
Moment arm

Abstract
The sarcomere length-joint angle relationship was measured in 7 different muscle-joint complexes (n = 43 muscles) of the frog hindlimb (Rana pipiens). Muscles studied included the cruralis, iliacus internus, gastrocnemius, gluteus magnus, gracilis major, semimembranosus and the semitendinosus. Muscle-joint complexes were mounted in a jig and submerged in chilled Ringer’s solution. Joints were rotated throughout their range of motion, while sarcomere length was measured by laser diffraction. Muscles were then formalin fixed and architectural properties determined by microdissection of individual muscle fibers. Sarcomere length change per degree of joint rotation (ΔL/Δθ) ranged from a low of 3.7 nm/degree for the cruralis muscle acting at the knee to a high of 12.5 nm/degree for the semitendinosus muscle acting at the hip. Values for ΔL/Δθ were significantly different between all muscles (p<0.001), and ΔL/Δθ values for muscles acting at the hip were significantly greater than those for muscles acting at the knee (p<0.005). ΔL/Δθ was negatively correlated with fiber length, suggesting a balance between fiber length and moment arm in most muscle-joint systems. However, many exceptions to this generalization were noted. These data suggest that different muscle-joint systems are ‘designed’ for differential contribution of muscle force production to the joint torque profile. The low variability of these data also suggests that sarcomere number is tightly regulated in these muscle-joint systems but not simply as a result of the total in vivo muscle excursion.

Introduction
Normal movement requires interaction between muscles and joints. Muscles generate force which is expressed as a joint torque based on the relationship between muscle force and moment arm. Recently, we detailed such a relationship in the frog semitendinosus hip and knee joint system. Specifically, the joint angle where maximum torque occurred was neither the joint angle at which the muscle developed maximum force nor the angle at which moment arm was maximum [1]. Further analysis revealed that the semitendinosus fiber length:joint moment arm ratio played a major role in determining the shape of the torque profile [2]. However, the extent to which this finding is applicable to other muscle-joint systems is not clear. Recently, Hoy et al. [3] demonstrated that the fiber length:moment arm ratio
could vary widely for several muscles of the human lower limb. Thus, the possibility exists that different muscle-joint combinations might be adapted in such a way that either fiber length or moment arm would dominate the shape of the torque profile.

Because the sarcomere length-joint angle relationship depends on both muscle fiber length and the moment arm, measurement of this parameter for various musculoskeletal units provides insight into the balance between muscle and joint properties. Therefore, the purpose of this study was to define the sarcomere length-joint angle relationship for a variety of frog hindlimb muscles in order to understand muscle-joint interaction more completely.

**Methods**

Seven different muscles from the hindlimb of the frog (Rana pipiens) were selected for study. This animal was chosen because of the frog's well-defined sarcomere length-tension relationship [4], which permits conclusions regarding muscle force generation to be made based on sarcomere length measurements. Hip flexors and hip extensors, knee extensors and knee flexors and an ankle extensor were studied (fig.1; table 1). Muscles studied included the crurals (n=8), iliacus internus (n=5), gastrocnemius (n=8), gluteus magnus (n=8), gracilis major (n=5), semimembranosus (n=4) and the semitendinosus (n=5). All muscles studied were biarticular with the exception of the iliacus internus.

**Muscle-Joint Preparation**

Frogs were sacrificed by double-pithing (n=43), and the selected muscle and associated joint(s) were isolated as previously described [1]. Care was taken to keep the hip and knee joint capsules intact and to maintain the complex in ice-cold Ringer's solution throughout the dissection. The appropriate portion of the skeleton (see below) was secured to a jig which enabled joint flexion and sarcomere length measurement. The entire jig containing the muscle-bone complex was mounted on a micromanipulator to permit positioning relative to the laser beam and immersed in chilled (12 °C) Ringer's solution composed of (mM): NaCl (115), KCl (2.5), Na2HPO4 (2.15), CaCl2 (1.8) and NaH2PO4 (0.85) adjusted to pH = 7.0. In this setting, the preparation was stable for 2-6 h.

**Sarcomere Length Measurements**

Sarcomere length was measured as a function of joint-angle by transilluminating the muscle with a 5-mW HeNe laser oriented perpendicularly to the long axis of the muscle. The laser beam was guided
using optical quality mirrors and attenuated as needed using a linear neutral density gray wedge (Model 4320; Melles Griot, Irvine, Calif., USA) mounted to a micromanipulator and fixed neutral density filters as needed. Sarcomere length was calculated from the distance between the zeroth and first-order diffraction lines using the grating equation:

\[ n\lambda = d\sin\theta, \]

where \( n \) = the diffraction order (1 in this case), \( \lambda \) = laser wavelength (0.632 \( \mu \)m), \( d \) = sarcomere length and \( \theta \) = diffraction angle relative to the undiffracted beam.

For all muscles except the semitendinosus, it was not possible to obtain diffraction patterns from the whole muscle due to either central tendons which scattered the laser or muscles with thickness greater than the laser could penetrate. As a result, muscles were dissected into smaller subunits to enable diffraction. Generally, these smaller subunits were composed of 10–20 fiber bundles composed of 25–50 fibers each which extended from tendon of origin to the tendon of insertion.

In order to determine whether the dissection procedure altered the sarcomere length measurements, we compared the sarcomere length-joint angle relationship of intact semitendinosus muscles to that obtained using dissected bundles from the same semitendinosus muscles. The average difference between whole-muscle measurements and measurements made from bundles obtained from the same whole muscle was 0.11 \( \pm \) 0.13 \( \mu \)m (mean \( \pm \) SD; \( n = 45 \) measurements from 5 different muscles) which was not significantly different from sarcomere length differences between various regions of the intact muscles (0.093 \( \pm \) 0.13 \( \mu \)m; \( n = 15 \) measurements from 5 different muscles). We thus concluded that sarcomere length-joint angle relationships from dissected bundles were equivalent to those of the intact muscle. (This result would not be true for most mammalian muscles with fibers that do not traverse from tendon to tendon.)

**Experimental Protocol**

For each muscle, sarcomere length was measured three times at 10° joint-angle increments yielding the sarcomere length-joint angle relationship (fig. 2). The range of motion studied varied for different muscles (table 1) and was that over which the muscle maintained low passive tension (so that tendon compliance would not complicate measurements [5]) with the opposing joint at 90°. This was also the range within which the joints operate physiologically during hopping [6]. However, for the gluteus maximus and cruralis muscles acting at the hip, the knee joint was flexed to 135°, for the gastrocnemius acting at the ankle, the knee was extended to 45°, and for the gastrocnemius acting at the ankle, the ankle was extended to 135° in order to prevent muscle sagging. The absolute sarcomere lengths thus depend on the relative hip and knee joint angles for the biarticular muscles, but sarcomere length change per degree of joint flexion (\( DL/\theta \)) would presumably not depend on the opposite joint angle due to the fact that the moment arm is small compared to the muscle length.

**Architectural Determination**

In addition to sarcomere length measurements on one side of the frog, contralateral muscles were dissected from origin to insertion and fixed in 10% buffered formalin for determination of muscle architectural properties according to a modification of the methods of Sacks and Roy [7]. Muscle length was measured as the distance from the most proximal fiber origin to the most distal fiber insertion. Nine single fibers (single fibers completely traversing from one tendon to the other) were dissected from the muscle (3 each from the ventral, middle, and dorsal regions), their fiber length (\( L_f \)) and sarcomere length (\( L_s \)) measured, enabling calculation of sarcomere number (\( n_s \), \( n = 27 \) samples/muscle) as:

\[ n_s = \frac{L_f}{L_s} \]

Muscle physiological cross-sectional area (PCSA) was calculated for each muscle [7] as:

![Fig. 2. Sample of the raw data obtained in the current study. Data shown were obtained from the gracilis major muscle while rotating the hip joint and maintaining the knee joint at 90° of flexion. Each symbol represents the average of 3 measurements from a single muscle. Linear regression of each data set was performed to yield slopes of 5.8, 7.9, 8.5, 10.3 and 6.1 nm/degree yielding an average of 7.8 \( \pm \) 1.8 nm/degree for this muscle-joint system.]

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Joints crossed</th>
<th>Range of motion studied (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cruralis</td>
<td>hip and knee</td>
<td>30– 80 (hip)</td>
</tr>
<tr>
<td>(( n = 7 ))</td>
<td></td>
<td>110–140 (knee)</td>
</tr>
<tr>
<td>Iliacus internus</td>
<td>hip</td>
<td>20– 65</td>
</tr>
<tr>
<td>(( n = 5 ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>knee and ankle</td>
<td>0– 80 (knee)</td>
</tr>
<tr>
<td>(( n = 8 ))</td>
<td></td>
<td>60–140 (ankle)</td>
</tr>
<tr>
<td>Glutaeus magnus</td>
<td>hip and knee</td>
<td>20– 55 (hip)</td>
</tr>
<tr>
<td>(( n = 6 ))</td>
<td></td>
<td>110–140 (knee)</td>
</tr>
<tr>
<td>Gracilis major</td>
<td>hip and knee</td>
<td>60–140 (hip)</td>
</tr>
<tr>
<td>(( n = 5 ))</td>
<td></td>
<td>110–140 (knee)</td>
</tr>
<tr>
<td>Semimembranosus</td>
<td>hip and knee</td>
<td>90–145 (hip)</td>
</tr>
<tr>
<td>(( n = 4 ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semitendinosus</td>
<td>hip and knee</td>
<td>30–140 (hip)</td>
</tr>
<tr>
<td>(( n = 5 ))</td>
<td></td>
<td>0–110 (knee)</td>
</tr>
</tbody>
</table>

\(^1\) Sample size.

Opposing joint at 90° unless otherwise stated in the text.
Table 2. Architectural properties of muscles studied

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Mass (mg)</th>
<th>Fiber length (mm)</th>
<th>Muscle length (mm)</th>
<th>PCSA (mm²)</th>
<th>Fiber:muscle length ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cruralis (n=7)</td>
<td>472±65</td>
<td>12.3±1.0</td>
<td>24.8±2.0</td>
<td>36.1±4.3</td>
<td>0.49±0.06</td>
</tr>
<tr>
<td>Iliacus internus (n=5)</td>
<td>58±15</td>
<td>14.5±1.8</td>
<td>21.1±1.7</td>
<td>3.9±1.5</td>
<td>0.69±0.12</td>
</tr>
<tr>
<td>Gastrocnemius (n=8)</td>
<td>546±14</td>
<td>9.5±0.6</td>
<td>29.0±2.5</td>
<td>54.7±14.0</td>
<td>0.32±0.03</td>
</tr>
<tr>
<td>Gluteus maximus (n=6)</td>
<td>151±38</td>
<td>15.6±1.4</td>
<td>21.6±2.3</td>
<td>9.1±2.2</td>
<td>0.72±0.03</td>
</tr>
<tr>
<td>Gracilis major (n=5)</td>
<td>349±89</td>
<td>20.3±0.9</td>
<td>30.7±3.0</td>
<td>16.3±0.8</td>
<td>0.66±0.03</td>
</tr>
<tr>
<td>Semimembranosus (n=4)</td>
<td>291±34</td>
<td>24.8±1.4</td>
<td>31.6±3.5</td>
<td>11.1±1.0</td>
<td>0.79±0.02</td>
</tr>
<tr>
<td>Semitendinosus (n=14)</td>
<td>45±10</td>
<td>10.5±1.4</td>
<td>22.5±1.7</td>
<td>3.51±1.3</td>
<td>0.46±0.04</td>
</tr>
</tbody>
</table>

Data are shown as means±SD. All fiber lengths are normalized to sarcomere length = 2.2 μm. n=Sample size.

¹ Data from Lieber et al. [5].

PCSA = \frac{(\text{muscle mass}) (\cos \theta)}{(L_o) (\rho)}

where \( \theta \) was pennation angle and \( \rho \) was muscle density (1.056 g/cm³ [8]).

**Statistical Analysis**

For each muscle, the sarcomere length-joint angle relationship was fit to a linear function yielding the dL/dθ. Sarcomere length-joint angle relationships over the entire range of motion were often curvilinear as previously demonstrated [1, 9]. However, for this study, we restricted our analysis to the linear portion of each curve which is similar to the physiological range observed during hopping [6]. The range over which this criterion was satisfied is shown for each muscle-joint combination in table 1. The linear fit was chosen instead of a more complicated model as had been used previously [9], to enable general comparisons between muscle-joint units. dL/dθ was compared between all muscles by one-way Anova. In order to compare average hip and knee slopes, a two-way Anova was used, grouping data by joint (hip or knee) and muscle. The significance level was set to \( \alpha = 0.05 \) with statistical power (1-β) calculated as 91%. Data were screened for normality and skew in order to justify the parametric methods used. Values in the text represent means±SEM unless otherwise noted.

**Results**

**Muscle Architectural Properties**

Muscles studied vary widely in terms of their absolute size (mass range from 58 to 546 mg) and design (table 2). The muscle with the largest cross-sectional area was the gastrocnemius (PCSA=546 mm²), while the smallest was the semitendinosus (PCSA=3.5 mm²). Based on the observation that PCSA is proportional to maximum tetanic tension [10], the gastrocnemius would be expected to generate over 150 times the force of the semitendinosus. The muscle with the longest fibers was the semimembranosus (Lₜ=24.8 mm), while the shortest fibers were measured in the gastrocnemius (Lₜ=9.5 mm).

**Sarcomere Length-Joint Angle Relationships**

All sarcomere length-joint angle relationships were well approximated by the linear fit since the lowest correlation coefficient (r) was 0.73, while the average for all data was 0.86. Sarcomere lengths measured in the midrange of the joint range of motion were generally on the descending limb of the sarcomere length-tension relationship [4]. Thus, for much of the normal joint range of motion, the muscles would be expected to produce less than maximum force. The relationship between sarcomere length and joint angle was significantly different among muscles crossing the hip (p<0.001) and knee (p<0.001) joints. For muscles crossing the hip, the dL/dθ ranged from a low of 6.2 nm/degree for the cruralis to a high of 12.5 nm/degree for the semitendinosus (fig.3). For muscles crossing the knee, dL/dθ ranged from a low of 3.7 nm/degree for the cruralis to a high of 9.3 nm/degree for the semitendinosus. Two-way Anova revealed a significant difference between dL/dθ for muscles crossing the hip compared with those crossing the
knee (p<0.005), a significant difference between muscles (p<0.001) with no significant interaction (p>0.8).

**Fiber Length-Sarcomere Length Interaction**

To understand the underlying basis for the particular sarcomere length-joint angle relationships measured, we investigated the correlation between dL/dθ and fiber length. There was a very weak negative correlation between fiber length and dL/dθ considering muscles crossing the hip and knee (fig.4). This relationship was stronger for muscles crossing the knee compared to muscles crossing the hip. However, within this generalization there was a great deal of variability. The largest dL/dθ for all muscles was seen for the semitendinosus acting at the hip (12.5 nm/degree). Other muscles with comparable fiber length were the gastrocnemius and cruralis (fig.4) which had significantly smaller dL/dθ (6.7 and 5.3 nm/degree, respectively). It follows that the semitendinosus moment arm is larger relative to its fiber length compared to either the gastrocnemius or cruralis. It is thus clear that short fibers are not necessarily associated with high dL/dθ as has been suggested [11].

Interestingly, dL/dθ measured during hip flexion was significantly greater than that measured during knee flexion (p<0.001). Average dL/dθ for all muscles crossing the hip was 8.3±2.9 nm/degree (mean±SD), while average dL/dθ for muscles crossing the knee was only 6.4±3.3 nm/degree. However, linear slopes of muscles crossing the same joint over a specified range of motion appeared to be similar though they were, in fact, significantly different (fig.5).

**Discussion**

The purpose of this study was to compare sarcomere length-joint angle relationships among several frog hindlimb muscles. Since this single parameter simultaneously reflects the balance between muscle fiber length (or, equivalently, sarcomere number) and muscle moment arm, it provides unique insights into muscle-joint interactions.

Sarcomere length-joint angle slopes (dL/dθ) varied significantly among muscles which crossed the same joint (fig.2) as well as among muscles crossing both the knee and hip joints. This result suggests that the fiber length:moment arm ratio is not simply adjusted so that sarcomere length-joint angle slopes are constant. Stated differently, sarcomere number is not simply determined by a muscle’s potential excursion. Clearly some muscle-joint systems have longer fibers relative to their muscle moment arms, while others have shorter fibers relative to their moment arms. This ratio has significant functional implications. For example, in recent studies of the semitendinosus muscle, we demonstrated that the sarcomere length change during hip joint flexion was 12.5 nm/degree [2] or knee joint flexion was 9.8 nm/degree [1], which resulted in an isotropic muscle force change of 80%, from 20% Pₒ at full extension to

---

*Fig. 3.* dL/dθ for the 7 different muscles studied. Note the variation in dL/dθ between muscles as well as general differences between muscles crossing the hip (■), knee (□) and ankle (■) joints. Data were not obtained for the gracilis major of semimembranosus acting at the knee.

*Fig. 4.* Relationship between muscle fiber length and dL/dθ. Short fiber length was negatively correlated with dL/dθ, but a great deal of variability was observed in this correlation. ○ = Muscles crossing the hip; ● = muscles crossing the knee; △ = ankle muscle.
100% P,

ear flexion. In contrast, the degree of force change during joint flexion would be much less in the cruralis, with its dL/dθ of only 3.7 nm/degree. Cruralis isometric muscle force would only change from 55 to 95% P,

if the knee were flexed from full extension to full flexion. These two examples represent the extremes obtained in the current study. Thus, in the same way that muscle architecture is a dominant factor which determines a muscle’s contractile properties [12], fiber length:moment arm ratio is the major determinant of the relative contribution of muscle and joint to the shape of the muscle-joint torque profile. While it is clear that the muscle force change throughout the entire range of motion is not a constant for different muscle-joint systems, it is possible that muscle force change over the portion of the range of motion in which the muscle is active is constant. Since the activation patterns in the frog hindlimb are not currently available, we cannot specifically address this possibility.

Most muscles studied crossed both the hip and the knee joint. In all cases, the slope measured during hip flexion was greater than that observed during knee flexion (fig.4). For the semitendinosus, the hip slope/knee slope ratio was 1.3. Thus, one might predict that during normal movement (while hip extension shortens the muscle and knee extension lengthens the muscle), since both the hip and knee are extending, the net result might be muscle shortening. This prediction does not hold true, however, when one considers that, during the frog hop, the knee joint extends approximately 120° while the hip joint extends only about 60° [6]. Therefore, during a normal hop, since the knee rotates twice as far, the net result is that muscle lengthening occurs [9]. We hypothesized that the knee extension moment which occurs during hopping is transferred to hip extension by the biarticular semitendinosus muscle. Preliminary studies on frog muscles during hopping support this concept [Olson et al., unpubl. data]. Whether this holds true for all biarticular muscles remains to be tested.

Finally, the data may have implications with respect to sarcomereogenesis during development. Sarcomere number is highly plastic, even in fully differentiated muscle [13, 14]. The fact that dL/dθ varies between muscles which cross the same joint implies that the muscle potential excursion is not the sole determinant of sarcomere number. Perhaps different muscles are recruited over different angles in the normal range of motion which permit them to regulate sarcomere number in response to tension, not length. Further studies which investigate the regulation of the sarcomere length-joint angle relationship during development and following chronic length change are required to define the factors which regulate sarcomere number.

![Graphical representation of the linear portion of the sarcomere length change for the range of motion studied.](image)

**Fig. 5.** Graphical representation of the linear portion of the sarcomere length change for the range of motion studied. a Muscles crossing the knee joint. b Muscles crossing the hip joint. Identical muscles in a and b are shown with the same symbols. Measurements were made with the opposing joint at 90° except that for the gluteus magnus and cruralis muscles acting at the hip, the knee joint was flexed to 135°, for the gastrocnemius acting at the ankle, the knee was extended to 45° and for the gastrocnemius acting at the knee, the ankle was extended to 135°.

**Acknowledgements**

The authors thank Christy Trestik for performing the architectural studies and Drs. Cyril Frank and Nigel Shrive (University of Calgary) and Dr. Kai-Nan An (Mayo Clinic) for helpful discussions. We also thank Rebecca Chamberlain for skillful artistic work. This study was supported by the Veterans Administration and NIH grant AR35192.
References


