SARCOMERE LENGTH MEASUREMENT PERMITS HIGH RESOLUTION NORMALIZATION OF MUSCLE FIBER LENGTH IN ARCHITECTURAL STUDIES

Amanda Felder, Samuel R. Ward and Richard L. Lieber
Departments of Orthopaedic Surgery and Bioengineering, University of California
and Department of Veterans Affairs Medical Centers, San Diego, CA; email: rlieber@ucsd.edu

INTRODUCTION

The reliability of architectural information depends heavily on accurate fiber length (L_f) values. One difficulty in obtaining accurate L_f values is compensating for the fiber length variation that occurs because muscles are fixed at various joint angles. The procedure typically used to compensate for such variation is to use sarcomere length measurements to normalize fiber length to a standard sarcomere length, using the equation:

$$L_f(cm) = \frac{L_f'(cm) \bullet L_s(\mu m)}{L_s'(\mu m)} \qquad \text{(Equation 1)}$$

where L_f is the normalized fiber length, L_f' is the experimentally measured (raw) fiber length, L_s is the standard sarcomere length and L_s' is the experimental sarcomere length measured at the experimentally measured fiber length. The purpose of this study was to test the accuracy and resolution of this method of fiber length normalization.

METHODS

The mouse hindlimb was used as a model system. Limbs were disarticulated at the hip, the knee joints were set to 90°, ankle joints were set to angles ranging from 30° to 150° (n=2-4/group), and the limbs were fixed in formalin. To determine the precise tibiotarsal and tibiofemoral angles of fixation, lateral radiographs were obtained of the limbs, and joint angles were digitized.

Tibialis anterior (TA), extensor digitorum longus (EDL), and soleus muscles were removed from limbs and digested in 15% H_2SO_4 to facilitate fiber bundle dissection. Small fiber bundles were dissected from the whole muscles under 8X-20X magnification, with care to remove the entire bundle from tendon to tendon. Three to four bundles were removed from each muscle. Fiber bundle length was measured to the nearest 0.01 mm. After mounting the fibers, sarcomere length was measured at three different points along each bundle using laser diffraction as previously described [1].

Raw fiber lengths were normalized to a standard sarcomere length of 2.5 µm using equation 1 shown above. Linear regression of raw and normalized fiber lengths over ankle angle was performed for each muscle. Additionally, raw and normalized fiber lengths were compared across angle groups by one-way analysis of variance (ANOVA). Finally, resolution of the normalization procedure was defined using the standard statistical power equations.

RESULTS AND DISCUSSION

As anticipated, raw fiber bundle length was strongly dependent on tibiotarsal angle (Fig. 1A; $p<0.002$ for all muscles, r^2 range from 0.20-0.68), although the magnitude of the change was muscle-dependent, based on the different fiber length-moment arm relationships of each muscle [2]. Sarcomere length normalization eliminated the joint-angle dependent variation in fiber length in all muscles, as evidenced by both linear regression (Fig 1B; $p>0.3$, r^2 range from 0.009-0.022) and one-way ANOVA ($p>0.1$). There was no significant variation of animal mass or tibial length across groups ($p>0.9$, $p>0.8$, respectively). A large degree of natural fiber length variation occurs which is not eliminated after sarcomere length normalization. Statistical power analysis revealed a ~90% chance of detecting a 15% fiber length difference and a ~60% chance of detecting a 10% variation.

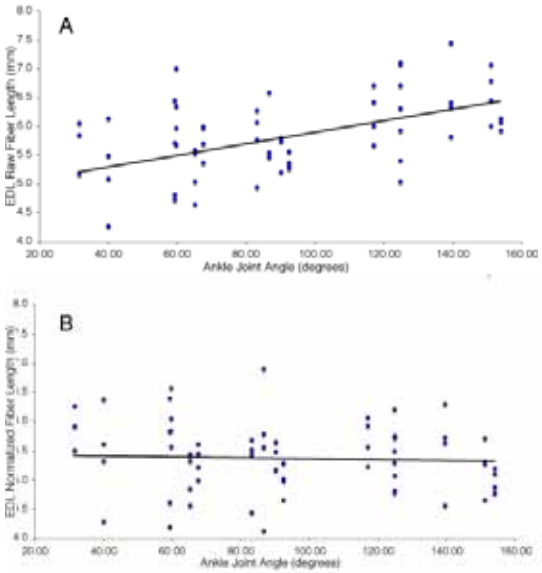

FIGURE 1: (A) EDL raw fiber length *vs*. ankle angle. (B) EDI normalized fiber length *vs*. ankle angle. Results from EDL are representative of TA and soleus as well.

CONCLUSIONS

We demonstrated that sarcomere length normalization eliminates the fiber length variability due to variation in joint angle. The use of sarcomere length normalization easily permits resolution of fiber length variations of 15%.

REFERENCES

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