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Short communication

Potential errors in fiber length measurements resulting from lever arm rotation during mechanical testing of muscle cells

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ABSTRACT

In single muscle cell preparations fibers are often suspended between connectors, extending perpendicularly from a force transducer, and the lever arm of a torque motor. The fiber does not move along a horizontal plane when shortened or lengthened by lever arm rotation. An error from the true length (*TL*) is introduced if the expected length (*EL*) is calibrated along this horizontal optical plane. Lever arm length (*LAL*), initial fiber length (FL_i), connector length (*CL*), and the magnitude of *EL* all contribute to this error. A mathematical model was used to determine the *TL* during shortening (0.96–0.80FL_i) and lengthening (1.10–1.50FL_i) at a constant *LAL* of 13.6 mm. *CL* had the greatest impact on error. For FL_i=2 mm at the longest *CL* modeled (15 mm), an expected shortening of 0.20FL_i produced a true shortening of $\sim$0.17FL_i, and an expected stretch to 1.50FL_i resulted in a true stretch to almost 1.60FL_i. Under these conditions, the true sarcomere length would be 4% and 6% longer than expected during shortening and lengthening, respectively. Because of their non-linear nature, length errors at long *CL*'s may result in an over-estimation of unloaded shortening velocity during slack tests and a left-ward shift in the passive tension-fiber length relationship at long fiber lengths. Measurement errors decreased dramatically with shorter *CL*'s, becoming negligible (< 1%) at *CL*=3 mm. We recommend that investigators keep *CL* as short as possible. Alternatively, we provide a method for adjusting the magnitude of the *EL* to yield a desired *TL*.

1. Introduction

Single muscle fiber preparations are important physiological tools for investigating relationships between protein expression and mechanical function (Reiser et al., 1985) and for understanding muscle mechanics in health and disease (Krivickas and Frontera, 2005; Malisoux et al., 2007). In a typical set-up the fiber ends are coupled to a force transducer and a length controller, and the fiber suspended in an experimental chamber mounted to the stage of an upright or inverted microscope. While some investigators utilize linear displacement transducers (Edman, 1979), many use high-speed rotational motors to rapidly and reproducibly alter fiber length (Julian and Morgan, 1979). In this latter type of arrangement, one end of the fiber is attached to a connector wire extending perpendicularly from the lever arm of the motor. The lever arm rotates to alter fiber length. However, this also displaces the fiber above or below the optical plane of the microscope. Because the length signal is normally calibrated along the optical plane, the fiber is actually lengthened more than intended during lengthening protocols and is shortened less than intended during shortening protocols. Our goal was to model the magnitude of the displacement error due to lever arm rotation in order to develop approaches for minimizing this error.

2. Methods

2.1. Geometric model

Fig. 1A illustrates a typical experimental arrangement for attaching a living or skinned muscle fiber segment to connectors extending from a position motor and a force transducer. The arrangement in Fig. 1A has been modeled geometrically in Fig. 1B for fiber shortening and in Fig. 1C for fiber lengthening. We assumed to know the lever arm length (*LAL*) given by *OH* and designated by *h*, the connector length (*CL*) given by *OB* and designated by *b*, and the initial fiber length (FL_i) given by *BF* and designated by *f* (Fig. 1, Table 1). By the Pythagorean theorem, one can calculate the radius *r* of *HB* and the length *d* between the axis of center *H* for the motor and the fixed transducer connector end *F* such that $r^2=h^2+b^2$ and $d^2=h^2+(b+f)^2$, respectively. When the connector is displaced, it makes a circular movement and the location of the left side of *BF* is changed from the point *B* to the point *P*, while the right side of the fiber is fixed at point *F* (Fig. 1B and C).

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