The Presence and Characterization of Force Depression in the *Drosophila* Jump Muscle

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***Abstract*—Force Depression (FD) describes the decrease in steady-state force production after active shortening in skeletal muscle. Despite being well characterized in skeletal muscle, the underlying mechanism remains unknown. The *Drosophila* jump muscle, tergal depressor of the trochanter (TDT), has recently been demonstrated to be mechanically similar to mammalian skeletal muscle, and due to the genetic simplicity of the organism, the TDT proves to be an excellent model to study FD. This investigation aimed to demonstrate both the presence of FD in the TDT, and that the phenomenon presented characteristically similar to skeletal muscle. In order to achieve this, wild-type Drosophila TDTs (n=10) were examined for FD in response to shortening amplitude (5, 10, 20% ML) and rate (4, 20, 200% ML/s). Results indicate that similar to mammalian skeletal muscle, TDTs exhibited an increase in the amount of FD with increasing amplitudes and decreasing rates of shortening.**

I. Introduction

The history-dependent phenomenon force depression (FD) is characterized by a decrease in isometric force production following active shortening. The amount of FD has been shown to increase with increasing amplitudes of stretch, decreasing rates of shortening, and increasing amounts of mechanical work [1]. Although FD has been demonstrated experimentally for over 70 years, and well characterized in whole [1,2] and single fiber [3] skeletal muscle preparations, the exact mechanism remains unknown. For that reason, it may be necessary to investigate FD in a new experimental animal model. *Drosophila* melanogastor, commonly known as the fruit fly, is a comprehensively understood and genetically manipulatable model organism. Furthermore, *Drosophila*'s flight muscles serve as an excellent model to studying muscle mechanics [4]. Recently, we have demonstrated the mechanical similarity of the Tergal Depressor of the Trochanter (TDT), or jump muscle, to mammalian skeletal muscle on the fiber level [5]. In addition, we have transgenetically exchanged sarcomeric proteins for their alternative isoforms, specifically myosin, and evaluated the kinetic and mechanical influences within the TDT muscle [5]. Due to the structural and mechanical similarities of the TDT muscle to skeletal muscle, as well as the potential use of genetic mutations in fly models, it is extremely advantageous to investigate the presence of history-dependent phenomenon in the TDT. If such phenomena are present, further investigations utilizing different myosin and actin isoforms could provide unique insight into the underlying mechanism(s) of this history-dependent phenomenon; insights which are impossible to elucidate using current experimental models. Thus, it is the goal of this study to determine the presence and characteristics of FD in the TDT muscle of wild type *Drosophila*. More specifically, we aim to determine if FD demonstrates the same characteristic relation to shortening amplitude and rate as observed in mammalian skeletal muscle.

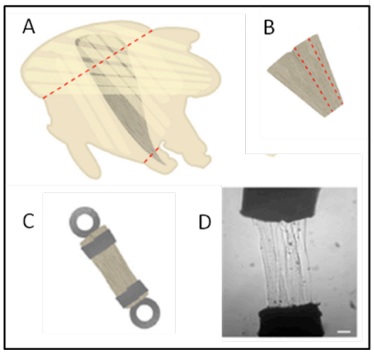

Figure 1. Schematic representation of TDT muscle preparation: (A) dissection of TDT from Drosophila thorax, (B) sectioning of fiber bundles, (C) T-clipped TDT fiber, and (D) clipped, mounted, and passively stretched muscle fiber (30 µm Scale Bar). Modified from Eldred et al. [5].

II. Methods

A. TDT Muscle Preparation

Transgenic Drosophila expressing wild-type myosin were created as described in Swank et al. [4]. Muscle fibers were dissected and mounted onto a fiber mechanics rig, as previously described [5]. In brief, TDT muscles were dissected from the thoraces of 2-3 day-old female *Drosophila* and chemically demembranated, or skinned, in dissection solution (5 mM MgATP, 1 mM Mg2+(free), 5 mM EGTA, 20 mM BES, 175 mM ionic strength, adjusted with Na methane sulfonate, 1 mM DTT, 0.5% Triton X-100, and 50% glycerol) for one hour at 4°C. Dissected fiber bundles were mounted upon a fiber mechanics rig with T-clips, laser cut from aluminum foil (MicroConnex). A schematic representation of TDT dissection, sectioning, and T-clipping can be seen in Fig.1. T-clipped fibers were evaluated for FD on a custom microscope-based, multi-welled single fiber mechanics rig, capable of measuring mN-scale loads with an AE-800 Series sensor element force gauge (SensorOne Technologies). Length manipulations were carried out at nanometer resolution and sub-millisecond response using a P-841.20 servo-motor (Physik Instrumente).

B. Isometric Contractions

TDT muscle fibers were mounted in relaxing solution (260

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mM ionic strength, adjusted with Na methane sulfonate, 10 mM MgATP, 45 mM creatine phosphate, 1200 U/ml creatine phoshokinase, 1 mM Mg2+ (free), 5 mM EGTA, 20 mM BES (pH 7.0), and 1 mM DTT) maintained at 15 °C. While in relaxing solution, TDT fibers were stretched to an average sarcomere length of 3.6 µm, as found previously [5]. Average sarcomere lengths and inter-clip dimensions were taken using a compound microscope and video analysis software (Ion Optix). TDT muscle fibers were transferred to preactivating solution (same as relaxing except: 0.5 mM EGTA) with an automatic well exchanger to help ensure sarcomere homogeneity during activation. After 2 min of equilibration in relaxing solution, TDT fibers were transferred to activating solution (pCa 5.0) where the isometric curves for the final length were recorded. After 60s of activation, TDT fibers were returned to relaxing solution for 5 min. Within relaxing solution, TDT muscle fibers were stretched to the starting length (20% of initial muscle length (ML) beyond 3.6 µm) and a similar set of procedures was carried out to obtain the isometric tension curve for the final shortened length.

C. *Evaluation of Force Depression*

A similar set of procedures was used for TDT muscle activation while investigating FD. However, rather than isometric evaluation, fibers were actively shortened during activation at three different rates (200, 20, and 4% ML/s), for the longest shortening amplitude (20% ML). Each fiber also underwent an active shortening of 20, 10, and 5% ML at a constant rate of 4% ML/s for a total of 5 experimental runs (n=10 per run). All shortening runs were 60s in total duration, and for each fiber, these experimental runs were systematically randomized. After two experimental runs, an isometric at the final length was taken to ensure the muscle was not significantly damaged or fatigued during the active shortening procedure.

III. Results

The investigation of force depression in wild-type myosin expressing TDTs has demonstrated not only the presence of FD in Drosophila TDTs, but also that it follows characteristically with the phenomenon observed in mammalian skeletal muscle. Specifically, the amount of FD increased with decreasing rates of shortening (Fig 2). Additionally, the amount of steady-state force depression increased with increasing amplitudes of shortening (Fig 3).

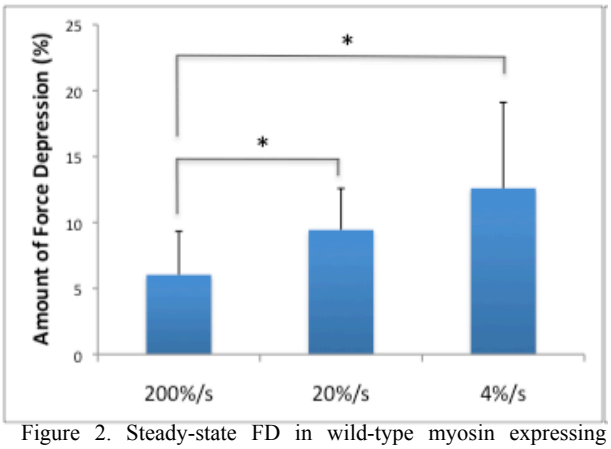

Figure 2. Steady-state FD in wild-type myosin expressing *Drosophila* TDTs showing an increase in FD with decreasing for rate of shortening. (n=10, * p<0.05)

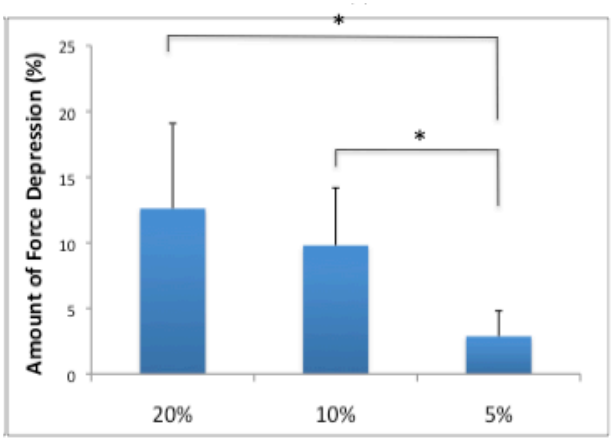

Figure 3. Steady-state FD in wild-type myosin expressing *Drosophila* TDTs showing an increase in FD with increasing amplitudes of shortening. (n=10, * p<0.05)

IV. Discussion

These studies are the first to establish the presence of force depression (FD) in *Drosophila* TDT muscle fibers. Moreover, the amount of FD was shown to scale with amplitude and rate of shortening (Fig. 2,3), mimicking the characteristics of this phenomenon in mammalian skeletal muscle [1]. These findings (a) demonstrate a presence of FD in *Drosophila* TDT muscle fibers, (b) correlate the FD similarities between the TDT muscle and skeletal muscle, and (c) imply additional history-dependent phenomenon such as force enhancement may exist. Future work will include a similar set of analyses on additional fly lines expressing variant isoforms of myosin and actin to uniquely explore the role of contractile proteins in FD, in order to better understand the kinetic and structural mechanisms involved in force depression and other history-dependent phenomena.

Acknowledgement

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