A new experimental model to study force depression: the *Drosophila* jump muscle

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Koppes RA, Swank DM, Corr DT. A new experimental model to study force depression: the *Drosophila* jump muscle. *J Appl Physiol* 116: 1543–1550, 2014. First published May 1, 2014; doi:10.1152/japplphysiol.01029.2013.—Force depression (FD) is a decrease in isometric force following active muscle shortening. Despite being well characterized experimentally, its underlying mechanism remains unknown. To develop a new, genetically manipulable experimental model that would greatly improve our ability to study the underlying mechanism(s) of FD, we tested the *Drosophila* jump muscle for classical FD behavior. Steady-state force generation following active shortening decreased by 2, 8, and 11% of maximum isometric force with increasing shortening amplitudes of 5, 10, and 20% of optimal fiber length, and decreased by 11, 8, and 5% with increasing shortening velocities of 4, 20, and 200% of optimal fiber length per second. These steady-state FD (FDSS) characteristics of *Drosophila* jump muscle mimic those observed in mammalian skeletal muscle. A double exponential fit of transient force recovery following shortening identified two separate phases of force recovery: a rapid initial force redevelopment and a slower recovery toward steady state. This analysis showed the slower rate of force redevelopment to be inversely proportional to the amount of FDSS, while the faster rate did not correlate with FDSS. This suggests that the mechanism behind the slower, most likely cross-bridge cycling rate, influences the amount of FDSS. Thus the jump muscle, when coupled with the genetic mutability of its sarcomere proteins, offers a unique and powerful experimental model to explore the underlying mechanism behind FD.

**force depression; history-dependent phenomenon; skeletal muscle; *Drosophila*; tergal depressor of the trochanter**

**IN RESPONSE TO ACTIVE shortening, skeletal muscle exhibits a reduction in force production, compared with an isometric contraction at the same final length, that cannot be explained by its force-length or force-velocity properties. This history-dependent behavior, known as force depression (FD), is a well accepted, yet poorly understood, property of skeletal muscle (1, 23). For nearly a century, scientists have studied the steady-state aspects of FD. Steady-state FD (FDSS) appears to be a sarcomeric phenomenon, since FDSS is present at all muscle structural levels: FDSS occurs in isolated single fibers (13, 17), and isolated single myofibrils (29, 39). FDSS is also observed when shortening occurs on the descending limb, the plateau, or ascending limb of the force-length curve (39, 41).

The classic characteristics of FDSS include greater FDSS with increasing amplitudes of shortening (9, 31, 34, 37), and lower FDSS with increasing shortening velocity (9, 21, 27, 31, 44). The amount of FDSS increases with larger magnitudes of force applied to the muscle during shortening (21) and increases with mechanical work done by the muscle during shortening (9, 21, 27, 30).

While most investigations have been limited to steady-state observations, a few recent studies have analyzed the transient force recovery that occurs immediately following active shortening to gain additional insight into FD (7, 9, 49, 50). In cat soleus muscles, analysis of the transient period following active shortening demonstrated a strong inverse correlation between the amount of FDSS and the rate of force redevelopment to steady state (9). More recent work in single fibers of the rabbit psoas muscle (7), pig urinary smooth muscle (49), and mouse soleus muscles (50) have demonstrated the need to fit the period of force redevelopment with a double exponential to accurately account for both a rapid and slower component of force recovery. Despite all of the previous studies focused on FD, the underlying mechanism(s) of FD remains unknown.

There are currently two predominant proposed theories to explain FD. Sarcomere nonuniformity theory links the depression in force to a nonhomogenous distribution of sarcomere lengths (SLs) after active shortening (13, 37). Alternately, stress-induced cross-bridge inhibition theory links FD to decreased cross-bridge binding due to stress-induced changes in actin filament structure (31, 34). While such theories hold great potential to explain the observed FD behavior, one reason for slow progress toward a proven mechanism is the lack of a model system with which we can easily manipulate sarcomeric protein expression to directly probe the underlying mechanism.

The *Drosophila* indirect flight muscle (IFM) fiber preparation, coupled with genetic manipulations, has been a very successful model system to study the structure and function of sarcomeric proteins and their influence on muscle phenomena, such as stretch activation (11, 45, 51). However, the IFM skinned fiber preparation generates low isometric tension (*F*iso) and cannot be used to study FD because its high passive stiffness makes for a relatively inextensible I band. This precludes conducting the large-amplitude length changes typical of a FD experimental protocol (6). Recently, we developed a new skinned fiber preparation utilizing an isolated bundle of fibers (8–10 fibers) from *Drosophila*’s tergal depressor of the trochanter (TDT), or jump muscle (14). Before this work, a mechanical analysis of the TDT was carried out using the entire TDT muscle (38), but we found that the whole muscle ripped easily and activated slowly (14). We showed that the jump muscle generates higher *F*iso, and the muscle’s more extensible I band allows for the long length changes required to perform force-length and force-velocity measurements. Thus we expect that the jump muscle will also allow for the long length changes required for FD measurements. If the jump muscle exhibits FD characteristics similar to those previously observed
in muscles from other organisms, our jump muscle preparation will be an outstanding, genetically malleable model organism for investigating FD mechanism(s).

To determine the extent and characteristics of FD in the jump muscle, we measured its force response to three amplitudes and three velocities of active shortening. We found that FD is present in the jump muscle. FDSS increases with increasing shortening amplitude and decreasing shortening velocities. These results mimic classic FD characteristics measured in other species. Similar to previous work (7, 49), we fit the transient period of force recovery following shortening with a double-exponential recovery function to accurately account for the biphasic response and measure both a fast and slow rate of redevelopment. The amount of FDSS correlated with a reduction of the slow recovery rate, indicating a change in cross-bridge cycling rate following active shortenings. This response is predicted by the proposed stress-induced cross-bridge inhibition mechanism of FD. Further tests of this mechanism, and other proposed FD mechanisms, are now possible at the protein structural level due to our unique, experimental model for FD.

MATERIALS AND METHODS

Fly line. The Drosophila line expressing transgenic wild-type myosin in its jump muscle was generated previously (47). The transgene contains genomic Mhc with flanking S' and 3' regions, including the Mhc promoter. When crossed with Mhc(0), null for myosin expression in the jump muscle, only the wild-type transgenic myosin is expressed. We used this specific transgenic fly line, rather than a pure wild-type line, because it establishes the foundation for future comparisons to transgenic lines expressing mutant myosin in the Mhc(0) background.

Jump muscle preparation and muscle testing apparatus. Muscle fibers were dissected and mounted onto a fiber mechanics apparatus, as previously described (14). Briefly, jump muscles were dissected from the thoraces of 2- to 3-day-old female Drosophila and chemically demembranated (skinned) in dissection solution for 1 h at 4°C. Dissected fiber bundles were then T-clipped and mounted on a custom-built, multwelled single-fiber mechanics apparatus. The apparatus is capable of measuring milli-Newton scale loads and performing length changes at nanometer resolution and sub-millisecond response.

FD measurements. To establish isometric traces for reference, jump muscle fibers were mounted in relaxing solution (260 mM ionic strength, adjusted with sodium methane sulfonate, 10 mM MgATP, 45 mM creatine phosphate, 1,200 U/ml creatine phosphokinase, 1 mM MgCl2 (free), 5 mM EGTA, 20 mM N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (pH 7.0), and 1 mM DTT) and maintained at 15°C. Jump fibers were stretched to an average SL of 3.6 μm, previously found to be on to the plateau of the tension-length curve (14), and the interclip length recorded as optimal fiber length (FLopt). Average SLs and interclip dimensions (length, width, and thickness) were measured with a compound microscope and calculated with video analysis software (Ion Optix). To achieve activation, fibers were transferred from relaxing solution to preactivating solution (same as relaxing, except EGTA is reduced to 0.5 mM) for 2 min, and then to activating solution (pCa 5.0) for 60 s, using an automated well exchanger to help ensure sarcomere homogeneity during activation. Isometric reference traces were recorded for both the longest starting length (1.2 FLopt) and final length (FLopt). Fiso was measured as the average of the last 3.0 s of the 60-s trace. Jump muscle fibers were returned to relaxing solution for 5 min after each activation to minimize deterioration (Fig. 1).

FD was characterized in jump muscles using a battery of concentric contractions from the descending limb to the plateau (FLopt). The muscle fiber was set at one of three starting lengths in relaxing solution. To calcium activate the fiber, it was moved first into preactivating solution and, then to activating solution. Once fully active, the fiber was shortened at a constant velocity of 4% FLopt/s to a consistent final length (FLopt, Fig. 1). To examine the dependence on shortening amplitude, each fiber was shortened by three different length change amplitudes, 5, 10, or 20% of FLopt, at the same velocity, 4% FLopt/s (Fig. 1). Similarly, to identify the influence of shortening speed on FD, each fiber was actively shortened over the largest amplitude, 20% of FLopt, at three different shortening velocities, 4, 20, and 200% FLopt/s, to FLopt. After shortening, muscle length was held constant for 30 s to allow force to recover toward a steady-state value, then the muscle was transferred to relaxing solution (Fig. 2). FDSS was found as the difference between the depressed steady-state force (FSS, Eq. 1) and the isometric force (Fiso) at the corresponding final length (FDSS = Fiso - FSS). When normalized to Fiso, this converts FDSS to a percent reduction in force.

An isometric reference contraction was collected at FLopt after every two active shortenings. The steady-state tension levels (FSS) of the two active shortenings were compared with this isometric contraction to calculate FDSS. This was done to limit any influence of preparation degradation on our FDSS calculations. Additionally, these isometric values were compared with the initial isometric reference traces to monitor the integrity and fatigue of the fiber. If more than a 30% decrease in force was observed between a reference isometric contraction and the initial isometric measurement, the fiber was deemed damaged and rejected from the study. All active shortening runs were systematically randomized from one experiment to the next to avoid order-dependent biasing. Muscle mechanical work done during shortening was calculated by integrating the force-displacement trace to find the area under the curve.

Transient analysis. Following active shortening, the data exhibit a biphasic force recovery consisting of both a rapid early recovery and a slower redevelopment to steady state. To quantify this transient force recovery immediately after shortening, the force-time data were analyzed using a double-exponential recovery function, similar to that of Van Noten and Van Leemputte (50). A double-exponential equation models this biphasic recovery well and accounts for the force transients described by Ford et al. (15). Specifically, the transient redevelopment period was analyzed using the following double-exponential function:

\[
F(t) = F_{ss} - (A_s e^{-kt_s} + A_k e^{-kt_k})
\]

where \( A_s \) and \( A_k \) are the amount of force recovery associated with both the fast and slow rate of recovery, respectively (i.e., the magnitude of force recovered during that phase), \( k_s \) and \( k_k \) are the fast and slow exponential force redevelopment rates, respectively (9), and \( t \) is time. Values for \( F_{ss} \), \( k_s \), \( k_k \), \( A_s \), and \( A_k \) were obtained using a
Levenberg-Marquardt error minimization algorithm in a commercial curve-fitting program (DeltaGraph 5.0, SPSS).

Statistical analysis. A block design, two-way ANOVA was employed to explore differences and determine statistical significance ($P < 0.05$). Regression analyses were used to determine the correlations between $FD_{SS}$, work, and the rates of force redevelopment ($k_F$,$k_S$).

RESULTS

$FD$ in Drosophila jump muscles. Jump muscle fibers exhibited classic $FD$ behavior (Fig. 2, A and B). The jump muscle produced $36.2 \pm 6.1 \, \text{mN/mm}^2 \, F_{\text{iso}}$ at $F_{\text{LOPT}}$. The $F_{\text{iso}}$ decreased (i.e., $FD_{SS}$) by 11, 8, and 5% following active shortening with increasing shortening velocities of 4, 20, and 200% $F_{\text{LOPT}}/s$, respectively (Table 1, Fig. 2C). Tension decreased by 2, 8, and 11% with increasing shortening amplitudes of 5, 10, and 20% of $F_{\text{LOPT}}$, respectively (Table 2, Fig. 2D). The greatest $FD_{SS}$ of 11% occurred at the largest shortening amplitude (20% $F_{\text{LOPT}}$) and the slowest velocity (4% $F_{\text{LOPT}}/s$). Mechanical work increased with the amplitude of shortening and decreased with velocity of shortening (Table 1). Regression analysis revealed that $FD_{SS}$ increased with the mechanical work done during shortening ($P < 0.05$, Fig. 3).

Transient force recovery. Analysis of the transient force recovery immediately following active fiber shortening revealed a biphasic response. To capture the biphasic nature of the data, a double exponential, $Eq. \, 1$ was fit to the force-time data following active shortening to capture both the fast and slow components of force recovery (Fig. 4). Fitting the data with a double exponential resulted in a better fit of the recovery traces ($R^2 = 0.983$) than a single-exponential fit to the entire force recovery trace ($R^2 = 0.908$).

Table 1. Influence of shortening velocity on transient and steady-state characteristics of force depression

<table>
<thead>
<tr>
<th>$FD_{SS}$, %</th>
<th>Work, nJ</th>
<th>$k_r$, s$^{-1}$</th>
<th>$k_s$, s$^{-1}$</th>
<th>$A_r$, mN $\times 10^2$</th>
<th>$A_s$, mN $\times 10^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% $F_{\text{LOPT}}/s$</td>
<td>11.02 $\pm$ 4.89</td>
<td>1.91 $\pm$ 0.91</td>
<td>0.70 $\pm$ 0.53</td>
<td>0.05 $\pm$ 0.04</td>
<td>1.94 $\pm$ 1.98</td>
</tr>
<tr>
<td>20% $F_{\text{LOPT}}/s$</td>
<td>8.21 $\pm$ 2.43*</td>
<td>1.62 $\pm$ 0.80</td>
<td>2.62 $\pm$ 2.22*</td>
<td>0.09 $\pm$ 0.06*</td>
<td>2.25 $\pm$ 1.62</td>
</tr>
<tr>
<td>200% $F_{\text{LOPT}}/s$</td>
<td>5.04 $\pm$ 2.14*</td>
<td>0.90 $\pm$ 0.75*</td>
<td>5.90 $\pm$ 2.44†</td>
<td>0.13 $\pm$ 0.07*</td>
<td>4.29 $\pm$ 1.58‡</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SD ($n = 10$). $FD_{SS}$, steady-state force depression; $k_r$ and $k_s$, fast and slow exponential force redevelopment rates, respectively; $A_r$ and $A_s$, amount of force recovery associated with both the fast and slow rate of recovery, respectively; $F_{\text{LOPT}}$, optimal fiber length. The amount of force depression ($FD_{SS}$), work, rate of force redevelopment ($k$), and the amount of force redevelopment ($A$) for three velocities of shortening are shown. Statistically significant ($P < 0.05$) from *4% and †20% $F_{\text{LOPT}}/s$ (two-way ANOVA).
The amount of force recovered for the fast redevelopment phase (A) appeared to decrease with increasing magnitudes of FDSS when FDSS was altered by changing velocity, but was independent of FDSS when FDSS was altered by changing amplitude (Tables 1 and 2). The amount of force recovered for the slower component of force recovery (A) did not appear to depend on FDSS when FDSS was altered by either experimental manipulation (Tables 1 and 2). Regression analyses of the pooled data of all FD tests performed on all fibers showed no correlation (P = 0.182) between FDSS and the fast redevelopment rate (kF) (Fig. 5A), but a significant (P < 0.001) negative correlation between FDSS and the slow force redevelopment rate (kS) (Fig. 5B).

Data grouped by (blocked for) either shortening speed or amplitude revealed a significant increase in kF with increasing shortening velocity (Fig. 6A, Table 1), and a slight negative correlation with increasing amplitude of shortening (Fig. 6B, Table 2). In contrast, kS was increased by both increased shortening velocity and decreased amplitude of shortening (Fig. 6, C and D, Tables 1 and 2).

### DISCUSSION

We found that the *Drosophila* jump muscle is an excellent experimental model to study FD, much better than the only other *Drosophila* muscle preparation, the IFM skinned fiber. The IFM has a short working length range and generates very low Fiso relative to other muscle types, which greatly restrict its ability to fully explore FD. We did not have this problem with the jump muscle, as we could perform length changes from 1% FLopt to 30% FLopt at velocities from 2% FLopt/s to 500% FLopt/s without tension levels dropping below zero. The jump muscle’s much higher Fiso generation, 20-fold, and lower passive tension and stiffness compared with IFM fibers (4, 14) makes for a better signal-to-noise ratio when measuring FDSS.

A slight disadvantage of the jump muscle is that a single-fiber preparation is very difficult to achieve. One factor is the small cross-sectional area of roughly 550 μm² (38). Instead of an isolated single fiber, we pare the muscle down to a bundle of 8–10 fibers. The fibers must be aligned in parallel when clamped in the T-clips to make certain no twisting occurs during contraction, to prevent tearing, and to ensure that all of the fibers are at the same SL. Jump muscle SL can easily be monitored, unlike the IFM, where SL measurement is difficult due to interference from trachea tubes running between IFM myofibrils. The SL of jump muscles is longer than vertebrates. A 3.6-μm starting length and a long plateau to the SL-tension curve (14) means we are operating at or near optimal overlap for almost all of the shortening runs. An additional disadvantage of working with skinned fiber preparations is that they are less structurally stable (3, 18, 19, 43) than intact muscle fibers (18). However, despite this challenge, the skinned jump muscle preparation proved sufficiently stable to allow several runs per fiber to be made without significant deterioration, such that 67% of jump muscle fibers tested fulfilled the inclusion parameters (as described in MATERIALS AND METHODS). These characteristics, along with a fairly easy and quick muscle preparation time of 3 h, make the jump muscle an attractive choice for studying FD.

The jump muscle demonstrated classic FD characteristics. Qualitatively, it displayed the typical increase in FDSS with decreasing velocities of shortening, increasing shortening amplitudes, and increasing muscle mechanical work (Figs. 2 and 3). Quantitatively, the *Drosophila* jump muscle fits well within the reported range of FDSS variation across species. The jump muscle exhibits a 7.8% FD for 10% shortening, a little less than a 1:1 ratio between FDSS and percent shortening. While this is a significant depression of force, some muscles exhibit even greater FDSS when actively shortened. An average of 30.9% FDSS for 14% muscle length shortening (≥2:1 ratio) was recently demonstrated in single isolated rabbit psoas myofibrils (28), and cockroach leg muscles showed an even greater amount, an average FDSS of 12% for muscle strain of only 2.5% (≥4:1 ratio) (2). FDSS in jump muscle was strikingly similar to previous findings in amphibian and mammalian muscle, at both the single-fiber and whole tissue level. Single fibers from frog lumbrical muscles exhibited FDSS of 9.3 and 14.7% for active shortening of 10 and 15%, respectively (~1:1 ratio) (31), and whole mouse soleus muscles showed a FDSS of 5.4, 9.5, and 16.3% for shortening of 5, 10, and 20% muscle length, respectively (~1:1 ratio) (50). This similarity to mammalian skeletal muscle, both qualitative and quantitative, further establishes the jump muscle as a good experimental model to study FD.

Our present study also provides insight into the nature of the FD mechanism because of our analysis of the transient force.

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Table 2. Influence of Shortening Amplitude on Transient and Steady-State Characteristics of force depression

<table>
<thead>
<tr>
<th>FDss, %</th>
<th>Work, nJ</th>
<th>kF, s⁻¹</th>
<th>kS, s⁻¹</th>
<th>A_F, mN × 10²</th>
<th>A_S, mN × 10²</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% FLopt</td>
<td>1.92 ± 0.91</td>
<td>0.61 ± 0.38</td>
<td>1.79 ± 1.81</td>
<td>0.10 ± 0.03</td>
<td>0.81 ± 0.39</td>
</tr>
<tr>
<td>10% FLopt</td>
<td>7.64 ± 3.56*</td>
<td>0.95 ± 0.78</td>
<td>1.38 ± 0.72</td>
<td>0.08 ± 0.03</td>
<td>0.87 ± 0.50</td>
</tr>
<tr>
<td>20% FLopt</td>
<td>11.02 ± 4.89*</td>
<td>1.91 ± 0.91*</td>
<td>0.70 ± 0.53*</td>
<td>0.05 ± 0.04*</td>
<td>1.94 ± 1.98</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 10). The amount of force depression (FDSS), work, rate of force redevelopment (k), and the amount of force redevelopment (A) for three amplitudes of shortening are shown. Statistically significant (P < 0.05) from *5% FLopt and †10% FLopt (two-way ANOVA).

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Fig. 3. The relationship between muscle mechanical work and steady-state force depression (FDSS) for all active shortenings. Regression analysis indicates a significant (P < 0.05) correlation between work and FDSS.
redevelopment. The need to study the transient force redevelopment following active shortening has been previously demonstrated (9), as has the biphasic nature of the force recovery period (7, 8, 49, 50). By utilizing a double-exponential curve to fit the transient recovery, we were able to capture both the fast and slow phases of force redevelopment and gain insight into which phase is more likely associated with the mechanism behind FDSS. The rapid component of redevelopment ($k_F$) in the Drosophila jump muscle did not correlate with FDSS when all shortening runs were pooled and plotted (Fig. 5A). This lack of correlation suggests $k_F$ is not linked to the FDSS mechanism. Instead, all observed changes in $k_F$ with amplitude and velocity (Fig. 6) can be explained by the basic muscle force-velocity relationship (16, 24). $k_F$ increased significantly with increasing rates of shortening (over the same amplitude). An increase in shortening speed results in greater unloading of the muscle (1, 33) and a higher proportion of negatively strained cross bridges (25). According to the Huxley model (25), the force redevelopment following shortening is determined by both the attachment rate of unbound cross bridges and the rate of detachment of negatively strained cross bridges, the later of which occurs much more rapidly. Thus one would expect a faster shortening to exhibit a quicker initial redevelopment of force (higher $k_F$). The much smaller effect of shortening amplitude on $k_F$ (Fig. 6B) is also expected. When comparing different shortening amplitudes at the same velocity, the muscle is under the same load during its shortening (due to its force-velocity relationship), and thus we would not expect a large change in the fast rate of force redevelopment.

Unlike $k_F$, the redevelopment rate of the subsequent slower phase, $k_S$, changed with both amplitude and velocity of shortening (Fig. 6). This would not be predicted from the force-velocity relationship because the amount of recovered $A_S$ did not change with increasing velocities (Table 1) or increasing magnitudes of shortening (Table 2). Instead, the results for the slow phase of force redevelopment are more likely influenced by, or indicative of, the mechanism responsible for FDSS because, when pooled for all active shortenings, $k_S$ negatively correlated with the amount of FDSS (Fig. 5B).

Ford et al. (15) described the mechanisms of the transient tension response following active shortening as a rapid change within attached cross bridges (myosin head rotation from weakly bound to strongly bound), followed by the release or detachment of cross bridges, and finally the attachment and detachment cycling of cross bridges as the force approaches steady state. Applying these myosin events to our transient FD analysis, our slow recovery rate, $k_S$, is primarily set by cross-bridges that are negatively strained. 

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**Fig. 4.** Representative force redevelopment traces following shortening fit with a double-exponential recovery function (Eq. 1) to account for both the fast ($k_F$) and slow components of force recovery ($k_S$), for three speeds of shortening, 4, 20, and 200% FL_{opt}/s (A), and three shortening amplitudes, 5, 10, and 20% FL_{opt} (B), illustrating that the biphasic force-time response is well described by the double-exponential function.

**Fig. 5.** The relationship between FDSS and both phases of force redevelopment, $k_F$ and $k_S$. Regression analyses across all active shortenings found no correlation ($P = 0.176$) between $k_F$ and FDSS (A) and a significant ($P < 0.001$) negative correlation between $k_S$ and FDSS (B).
bridge cycling rate. Since our slow recovery phase was more closely associated with $F_{DS\text{SS}}$, this likely implies that alterations in attachment and/or detachment rates are an important component of the FD mechanism.

There are currently two predominant proposed mechanisms for FD. At present, the most widely accepted mechanism for FD is SL nonuniformity theory, in which the reduction in $F_{DS\text{SS}}$ is attributed solely to the development of large dispersions in SLs during active shortening (13, 36, 37). This mechanism, as we understand it, does not lead to a clear prediction of how rates of force regeneration following active shortening should vary under different shortening conditions. However, an alternate FD theory, the stress-induced cross-bridge inhibition mechanism (34) does predict how rates of force regeneration should be influenced. This model for FD, originally proposed by Marechal and Plaghki (34), and furthered by Herzog (20), hypothesizes that active shortening causes stress-induced deformation of actin filaments, which, in turn, leads to a lower number of strongly bound cross-bridges and a reduction in $F_{DS\text{SS}}$. More specifically, the theory proposes that actin deformation, when subjected to physiological loads (22, 26), will be much smaller in the areas reinforced by attached cross bridges before shortening (i.e., the original overlap zone). The remaining length of actin outside of the A band, not reinforced before shortening, will deform due to the load on the muscle, and this deformation can be enough to significantly decrease the binding probability of cross bridges. During muscle shortening, portions of the deformed actin are brought into overlap with myosin, a new overlap zone, and it is in this zone that cross-bridge binding rate would be decreased (20, 34). Greater amplitudes of shortening increase the length of the new overlap zone, while slower speeds increase the deformation of actin due to greater force on actin. Both of these situations would decrease myosin attachment rate, which would be predicted to decrease $k_s$ because (according to Ref. 15) the final force redevelopment phase [our slow component, $k_s$; phase 4 of Ford et al. (15)] is partly dependent on myosin attachment rate. We observed the predicted decrease in $k_s$ with both increased amplitudes of shortening (Fig. 6D) and decreased velocity of shortening (Fig. 6C) in our jump muscle experiments. Therefore, our force redevelopment observations support the stress-induced inhibition mechanism.

Our $F_{DS\text{SS}}$ results from varying length change and velocity also fit into the stress-induced inhibition mechanism. Higher forces on actin during shortening, due to slower speeds of shortening, are predicted to create larger distortions of actin, causing a greater inhibition of cross-bridge formation. This leads to fewer total cross bridges bound, resulting in greater $F_{DS\text{SS}}$. Likewise, larger shortenings would create a longer new overlap zone and would increase $F_{DS\text{SS}}$ by affecting a greater number of cross bridges. We observed both of these predictions with Drosophila jump muscle, as it exhibited increased $F_{DS\text{SS}}$ with slower shortening (Fig. 2C) and larger amplitudes of shortening (Fig. 2D).

The Drosophila jump muscle is an excellent experimental model to test the stress-induced cross-bridge inhibition FD model. We can harness the power of Drosophila genetics, such as P-element-mediated transformation, nulls, and conditional gene expression systems, to manipulate the protein components of the sarcomere (35, 42). For example, we can express any Drosophila myosin isoform we wish in the jump muscle (45, 52) or make chimeras of isoforms (46) or specific mutations in isoforms (40). Thus we can likely change myosin attachment or detachment kinetics to probe the mechanism behind FD. Actin could be mutated to change its compliance (12). This might be a good test of the stress-induced inhibition model because it predicts that, as actin compliance is increased, $F_{DS\text{SS}}$ should increase and $k_s$ decrease. Thus our new experimental model, the jump muscle, combined with the power of Drosophila genetics, should continue to provide new insight into the underlying mechanism of FD.
REFERENCES


