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Measured and modeled properties of mammalian skeletal muscle: III. the effects of stimulus frequency on stretch-induced force enhancement and shortening-induced force depression

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Abstract

Stretch-induced force enhancement and shortening-induced force depression were examined in fast-twitch feline caudofemoralis muscle at 37°C. These phenomena were induced by applying ramp length changes during the first 100–200 ms of an otherwise isometric contraction. The effects of various stimulus frequencies ranging from 30 to 120 pps were investigated over lengths ranging from 0.85 to 1.15 L_0. Distributed asynchronous stimulation of bundles of ventral roots was employed to produce smooth contractions at sub-tetanic stimulus frequencies in whole muscle. Of the two components of force enhancement identified by Noble (1992) we observed only the transient component that decays with time; we did not observe residual force enhancement. The force depression that we observed was symmetrical in almost all respects to the transient force enhancement, and was unlike the shortening-induced de-activation and residual force depression identified by Edman (Edman, 1975; Edman *et al.*, 1993). Both transient force enhancement and depression were independent of work, load and activation. Reversals in the direction of ramp length changes following either an initial stretch or initial shortening were shown to cancel the effects of both transient force enhancement and transient force depression. The distances over which these cancellations could be achieved were different for the lengthening and shortening effects. This asymmetry can be reconciled with the predictions of Huxley's original cross-bridge mechanism by incorporating the recent suggestion that myosin heads can interact with multiple actin binding sites during a single 'working' stroke. We conclude that the types of force enhancement/depression that are most likely to be encountered under physiological conditions are the transient effects observed here, but that even these will have relatively little effect on force production during most natural behaviors.

Introduction

It has long been known that recent kinematic history plays an important role in skeletal muscle force generation (e.g. Fenn, 1924; Abbott and Aubert, 1952). Stretch prior to isometric activation can lead to subsequent force enhancement (Edman *et al.*, 1978, 1981, 1982; Julian and Morgan, 1979; Sugi and Tsuchiya, 1988) whereas prior shortening can lead to force depression (Edman, 1975, 1980; Maréchal and Plaghki, 1979; Granzier and Pollack, 1989; Edman *et al.*, 1993; Herzog and Leonard, 1997). These phenomena are intriguing because they promise to shed light on the details of the contractile mechanisms.

Despite a long history of investigation, however, the mechanisms responsible for these phenomena are still somewhat controversial (e.g. Noble, 1992; Herzog and Leonard, 1997). Part of the reason for this controversy stems from the fact that both stretch-induced force enhancement and shortening-induced force depression (hereafter called simply force enhancement and force depression) each appear to have at least two independent components (Noble, 1992; Edman *et al.*, 1993). Many previous investigators apparently have not recognized this fact and instead attempted to account for all force enhancement observations or force depression observations by a single mechanism. As a result they may have prematurely discarded valid hypotheses to explain the individual components of force enhancement or force depression, resulting in the present controversy.

In this study, we examined the interactions between applied length and velocity conditions during relatively brief periods of activation at various physiological frequencies. These conditions are different from those employed in many previous studies of these phenomena but they were designed to meet our objective of quantifying the contractile properties of muscle under conditions relevant to physiological use. We used a whole-muscle preparation at normal body temperature

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with asynchronous stimulation of bundles of motor axons. This is the only method of activation *in vivo* that can simulate the sub-maximal but smooth contractions that occur normally when motor units are recruited physiologically.

In continuation of a series of previous studies (Brown and Loeb, 1998, 1999; Brown *et al.*, 1999) we conducted these experiments in feline caudofemoralis (CF) muscle. CF was particularly suitable because it has a simple, parallel-fibered architecture with no pennation angle and an almost exclusively fast-twitch fiber composition (95–100%; Brown *et al.*, 1998). CF also has a high ratio of fascicle length to aponeurosis length (~5:1), which results in a whole-muscle preparation with very little in-series compliance (Brown *et al.*, 1998), permitting applied length and velocity conditions to be transmitted faithfully to muscle fibers. The potentiated state was chosen as the base state for our data collection following a previous study in which we concluded that the normal operating state of fast-twitch muscle is, in fact, the potentiated state (Brown and Loeb, 1998). We based this conclusion on the following observations: after a few sub-tetanic trains, a stable and near maximal level of potentiation can be reached; this stable level of potentiation can be maintained with low-intensity, intermittent activation; this stable level of potentiation disappears only after a long period of inactivity. Throughout this paper the phrase 'potentiated state' refers to this stable near-maximal level of potentiation.

Materials and methods

Experimental data collection

The experimental apparatus and procedures used in this study were similar to those described previously for feline CF muscle (Brown and Loeb, 1998; Brown *et al.*, 1999). Experiments were carried out in seven cats of either sex (2.6–5.4 kg) under deep pentobarbital anesthesia as determined by the absence of pedal withdrawal. Briefly, the feline CF muscle was dissected free of surrounding tissue with its origin, innervation and blood supply left intact. It was maintained thermostatically at $37 \pm 1^{\circ}C$ in a mineral oil pool. The length of the muscle fascicles was controlled by clamping onto caudal vertebrae Ca2 and Ca3 (CF origin) and onto the insertion tendon at the point where the distalmost fibers terminate. This eliminated virtually all series-compliance in the linkage. The insertion clamp was attached to a computer-controlled muscle puller via a force transducer. Electrical stimulation was applied via platinum–iridium hook electrodes on cut L7 and S1 ventral roots and monitored by recording M-waves via two multi-stranded stainless steel wire electrodes inserted transversely through the muscle approximately 5 mm apart. Computer templates controlled the length, velocity and stimulus patterns simultaneously for long preprogrammed sequences defining entire experimental protocols in 1.667 ms steps. During each step, the computer program recorded values for both the force and a rectified, bin-integrated representation of the M-waves. Force was digitally filtered after the experiment using a double-pass, second-order Butterworth filter with a 3 dB cutoff frequency of 120 Hz to remove high-frequency (150–200 Hz) resonant noise from our system.

To produce smooth contractions at sub-tetanic stimulus frequencies, the ventral roots were split into five bundles that were stimulated asynchronously. The maximal dispotentiated twitch force produced by each individual bundle ranged from 15–25% of the maximal dispotentiated twitch force produced by the whole-muscle. In this study, stimulus frequency always refers to the frequency applied to each of the nerve bundles.

At the beginning of each experiment passive and tetanic force-length (FL) curves were collected in the dispotentiated state. The dispotentiated state was maintained by inserting 5 min passive intervals between each stimulus train (Brown and Loeb, 1998). Passive force data were collected in the isometric state 30 ms after stretching to at least 15 different lengths. These passive forces were then subtracted post-experiment from all subsequent force records to provide an estimate of active force. Passive forces were minimal in these experiments, less than 5% of maximal isometric tetanic force at the longest lengths tested. Preliminary estimation of L_0 for the tetanic FL curves was made based upon *in situ* anatomical lengths (Brown *et al.*, 1998). Based on this estimate, isometric tetanic contractions (120 pps, 15 p trains) were elicited at 0.7, 0.8, 0.9, 0.95, 1.0, 1.05, 1.1 and 1.2 L_0. The 'true' L_0 was then chosen as the length at which maximal tetanic isometric force (F_0) could be elicited. Sarcomere length measurements collected during these experiments demonstrated that the peak of the tetanic FL relationship occurs reliably at ~2.4–2.5 μm, as expected based upon sliding filament theory. These data have been published previously as part of a larger study on CF morphometry (Brown *et al.*, 1998).

All other stimulus paradigms in this series of experiments were conducted while CF was potentiated. These paradigms used stimulus trains that were always separated by 7 s. Potentiation was achieved by applying twelve isometric 10 p trains at 60 pps at three successive lengths (0.75, 0.95 and 1.15 L_0) and maintained with 15–120 pps stimulus trains (8–12 p duration). This protocol has been shown previously to provide a stable and apparently near-maximal level of potentiation in CF (Brown and Loeb, 1998). The muscle and nerve preparations were allowed to relax for at least 45–60 min prior to data collection. In all paradigms stimulation voltage (0.2 ms rectangular pulses) was five times higher than the threshold required to elicit an M-wave. M-wave amplitude was monitored continuously to ensure complete recruitment of the entire muscle throughout the experiment. We applied single isometric twitches at 1.0 L_0 one second before specified stimulus trains so that peak twitch force could be used to monitor potentiation (Brown and Loeb, 1998).

Results

We employed several different paradigms to examine the effects of changes in length and velocity on subsequent force production. In the first two of these paradigms we imposed brief ramp length changes at the beginning of the stimulation train and looked at the effects on isometric force during the remainder of the contraction. The first of these paradigms used ramp length changes of ± 1.0 L_0/s for 100 ms and two sets of stimulus conditions: 120 pps at 0.85 L_0 and 30 pps at 1.0 L_0. Sample data traces are shown in Figure 1A. The second of these paradigms used ramp length changes of ± 0.5 L_0/s for 200 ms and four sets of stimulus conditions: 50 pps at 0.85 L_0 and 30 pps at each of 0.85, 1.0 and 1.15 L_0. Sample data traces are shown in Figure 1B. Initial stretches always resulted in greater force during the subsequent isometric period whereas initial shortening always resulted in less force.

We quantified the effects of force enhancement/depression in these two paradigms by calculating the ratio of stretch-enhanced/shortening-depressed force vs. control isometric force 100 ms after the end of each length change (vertical dashed lines in Figure 1). The mean values for the ± 1.0 L_0/s data and the ± 0.5 L_0/s at 0.85 L_0 are plotted vs. work done by/on the muscle in Figure 2. Paired t-tests showed no significant effect in either of these paradigms, implying that the force enhancement and force depression observed here were

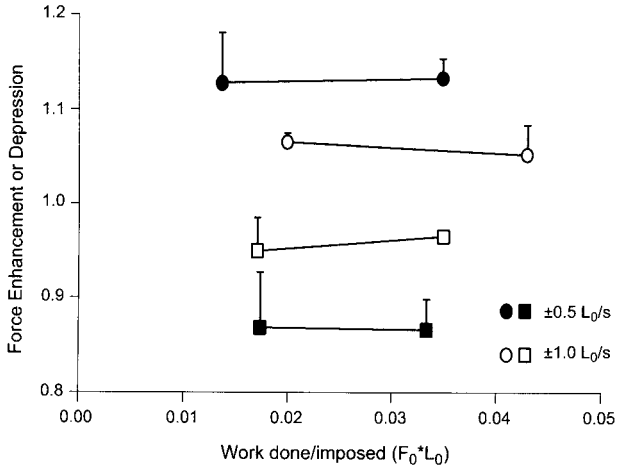

Fig. 2. Relationship between work and force enhancement/depression. Mean ± SD values of force enhancement/depression are plotted as a function of work done on/by the muscle. Comparisons between 30 and 120 pps data (± 1.0 L_0/s for 100 ms) and between 30 and 50 pps data at 0.85 L_0 (± 0.5 L_0/s for 200 ms) revealed no significant difference (Paired t-tests, $n = 3$; for both force enhancement and depression: $P > 0.4$ for ± 1.0 L_0/s data and $P > 0.9$ for ± 0.5 L_0/s).

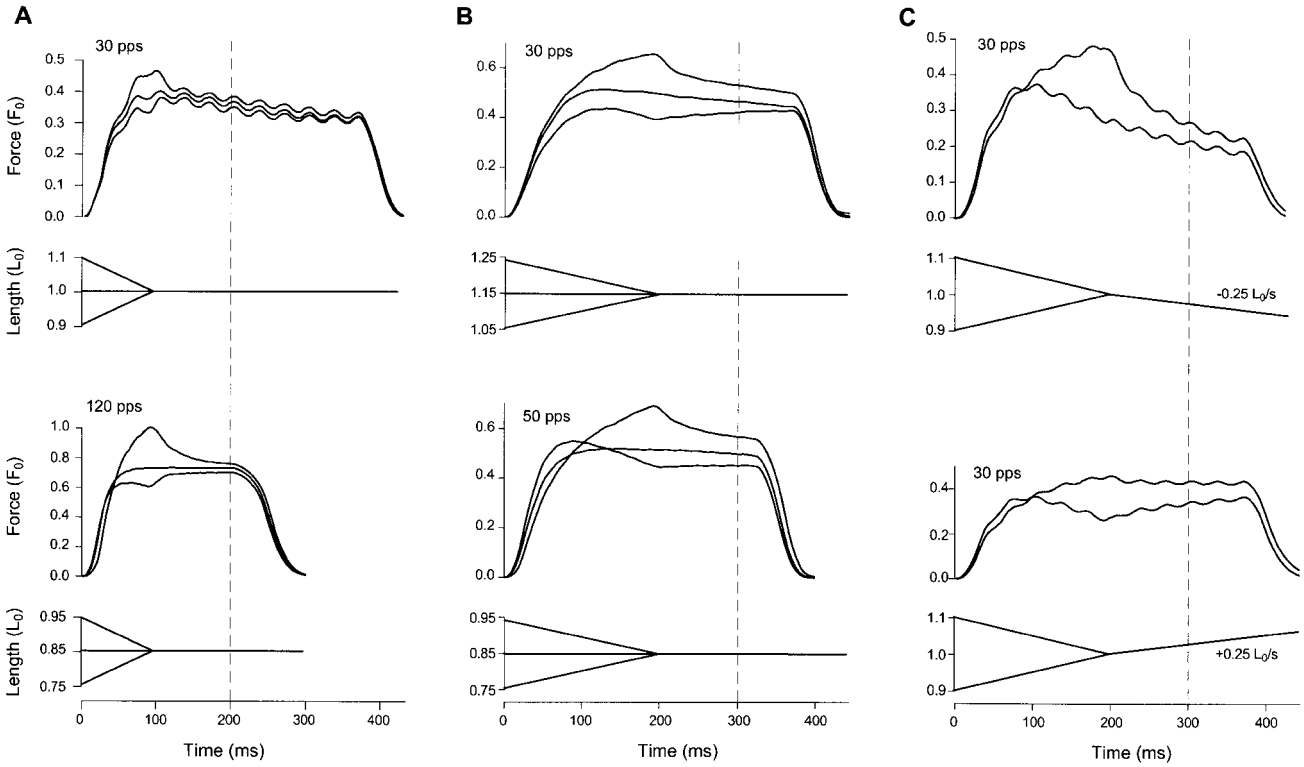

Fig. 1. Stretch-induced force enhancement and shortening-induced force depression. (A, B) Sample data traces demonstrating transient force enhancement or depression during isometric conditions following length changes of 100 ms at ± 1.0 L_0/s (A) or 200 ms at ± 0.5 L_0/s (B). Initial stretches always resulted in greater force during the subsequent isometric period whereas initial shortening always resulted in less force. Force enhancement/depression 100 ms after the length change (dashed lines) were compared statistically for various stimulus conditions as described in text. (C) Sample force traces demonstrating transient force enhancement or depression during slow length changes (± 0.25 L_0/s) following an initial 200 ms length change at ± 0.5 L_0/s. Force enhancement/depression at $t = 300$ ms (dashed lines) were compared statistically as described in text.

independent of work, load and activation (for both force enhancement and depression: $P > 0.4$ for $\pm 1.0\ L_0$/s data and $P > 0.9$ for $\pm 0.5\ L_0$/s).

The mean values for the $\pm 0.5\ L_0$/s data were plotted vs. length (Figure 3) to determine if there was an effect of length on either force enhancement or force depression. For statistical analysis each value was normalized to the mean enhancement/depression for each animal to remove inter-animal variability. A weak but statistically-significant, positive linear correlation between length and force enhancement was observed ($P < 0.01$), whereas a weak but statistically-significant parabolic correlation was observed between length and force depression ($P < 0.05$).

In three experiments we examined the additional effect of imposing ramp length changes of $\pm 0.5\ L_0$/s for 200 ms prior to starting stimulation (30 pps at $1.15\ L_0$, data not shown). These passive length changes produced nearly-negligible but statistically-significant effects (force enhancement 100 ms after the length change was 1.029 ± 0.002 and 1.015 ± 0.003 for the stretching and shortening conditions respectively; paired t-test, $P < 0.02$).

To test the hypothesis that the force enhancement/depression described above persist only as long as the muscle remains isometric, similar experiments were performed in which the 200 ms length changes at $\pm 0.5\ L_0$/s were followed by either an isometric period or by slow length changes at $\pm 0.25\ L_0$/s. Sample data traces for this paradigm are shown in Figure 1C (isometric traces not shown because they are similar to those shown in Figure 1B). This paradigm was conducted only at 30 pps, $1.0\ L_0$. The mean ratio of force enhancement to force depression from three experiments is plotted in Figure 4A for each of the three conditions. The time course of decay is similar for all three conditions. The mean ratios at $t = 300$ ms during imposed length changes of $+0.25$, 0 and $-0.25\ L_0$/s were not significantly different from each other (repeated-measures analysis of variance, $n = 3$, $F = 2.16$, $P > 0.23$). For the trials in which subsequent length changes at $\pm 0.25\ L_0$/s were involved, the ratios of force enhancement to force depression plotted in Figure 4A were re-plotted vs. estimated half-sarcomere length changes in Figure 4B. Extrapolation suggests that the difference between force enhancement and force depression would decay to zero after length changes of approximately 100 nm per half sarcomere in both conditions.

The fourth paradigm that we used in this series looked at the effects of reversing the direction of ramp length changes to cancel force depression or force enhance-

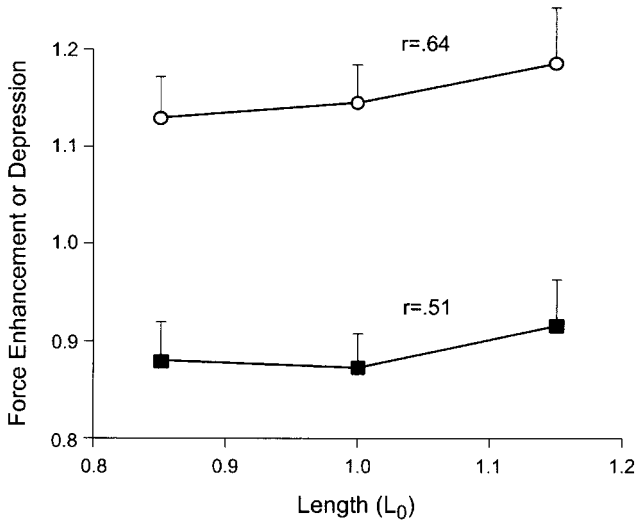

Fig. 3. Length dependence of force enhancement and force depression. Mean ± SD values of force enhancement/depression are plotted as a function of length ($n = 8$, 4 and 4 for 0.85, 1.0 and 1.15 L_0 respectively, 30 and 50 pps data at 0.85 L_0 included together). As a first approximation to removing inter-animal variability, correlation values were based upon scores that had been normalized to the mean force enhancement/depression for each animal. Force enhancement was correlated positively with length ($r = 0.65$, $P < 0.001$) whereas force depression was correlated parabolically with length ($r = 0.51$, $P < 0.05$).

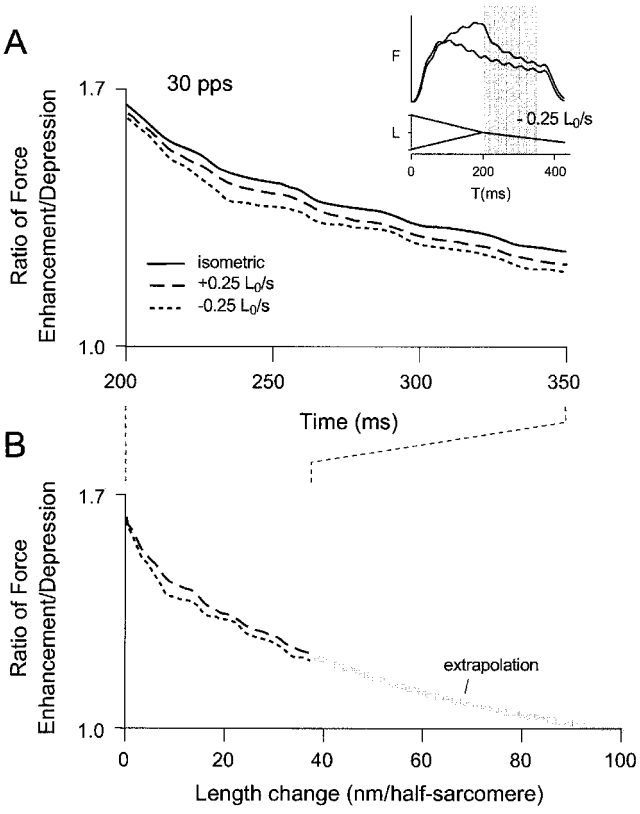

Fig. 4. Ratio of force enhancement to force depression. (A) The ratio of force enhancement to force depression following 200 ms length changes at $\pm 0.5\ L_0$/s was calculated during three subsequent kinematic conditions: $+0.25\ L_0$/s, isometric and $-0.25\ L_0$/s. Sample force and length traces are shown in the inset figure (also shown in Figure 1C); the shaded region represents the times for which the ratio was calculated. The mean ratios for three experiments are plotted here. The time course of decay is similar for all three conditions. (B) For the $\pm 0.25\ L_0$/s traces in A we re-plotted the ratio of force enhancement to force depression vs. length change in units of nm per half-sarcomere. The shaded line shows the estimated extrapolation to zero force enhancement after approximately 100 nm of length change.

ment. All velocities used in this paradigm were $\pm 1.0\ L_0$/s (this paradigm is essentially identical to the one shown in Figure 5 of Edman, 1980). Stimulus trains were at 30 pps at 1.0 L_0. Sample force and length traces are shown in Figure 5A and 5B along with a reference fully isometric trial (dashed trace). The force enhancement from an initial stretch shown in Figure 5A disappeared quickly during subsequent shortening. Similarly the force depression from initial shortening in Figure 5B disappeared quickly during subsequent lengthening. The time course of these effects is seen more clearly in Figure 5C in which the relative force enhancement between the two non-isometric trials is plotted vs. time. Almost all of the extra force enhancement from stretch disappeared in the first 20 ms of subsequent shortening. In contrast it took approximately 50 ms for the force depression to disappear during subsequent lengthening. If we plot the decay of force enhancement vs. half-sarcomere shortening distance instead of time (second axis in Figure 5C), the initial decay of force enhancement extrapolates to zero for a shortening distance of approximately 15 nm per half-sarcomere (assuming sarcomere L_0 is 2.4 μm; Herzog *et al.*, 1992). For force depression the corresponding lengthening distances are much greater, approximately 40–50 nm per half-sarcomere.

Discussion

As mentioned in the introduction, much previous work investigating the phenomena of stretch-induced force enhancement and shortening-induced force depression has been confused by the existence of least two independent components of each. We suggest below that there is, in fact, a third component of force depression that apparently has not been observed in single fiber preparations at cold non-physiological temperatures. Furthermore, we believe that two of the three components of force depression are symmetrical with the two components of force enhancement and that all of these components can be explained via previously proposed hypotheses.

To organize this discussion we have summarized our findings in relation to those of many previous studies in Table 1. We have labeled the various components of force enhancement and force depression as either transient or residual, based upon their duration. Because some previous authors did not clarify which component they observed (e.g. Sugi and Tsuchiya, 1988), we carefully examined the published data records in each

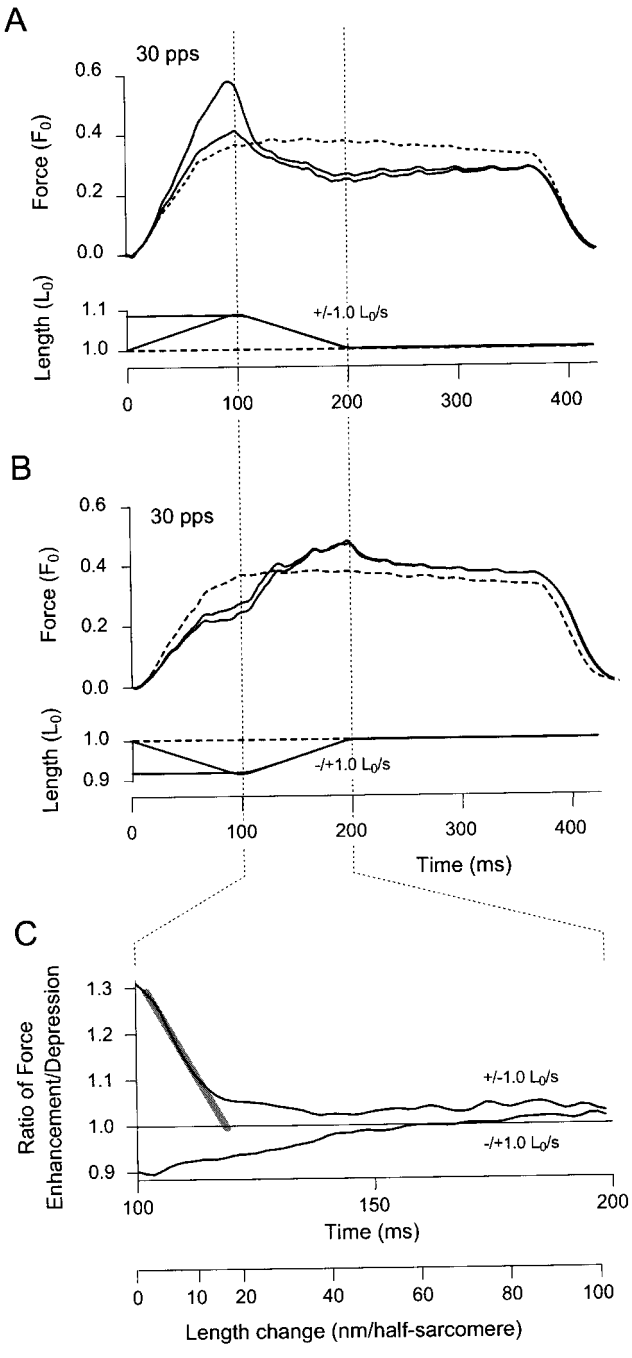

Fig. 5. Velocity reversals and their effect on transient force enhancement/depression. (A, B) Sample force and length traces demonstrating the decay of force enhancement during subsequent shortening (A) or the decay of force depression during subsequent lengthening (B). All velocities were $\pm 1.0\ L_0$/s. In both figures the isometric case is shown as a dashed trace for reference comparison. In A the initial stretch produced greater force than the initial isometric trial, whereas in B the initial shortening produced less force than the initial isometric trial. (C) The relative amount of force enhancement or depression in A and B was estimated by dividing the forces from the trial with an initial length change by the corresponding forces from the trial with initial isometric conditions (the purely isometric trials were not used in these calculations). The mean values of relative force enhancement/depression from two animals are plotted here for $t = 100$–200 ms. (Qualitatively similar results from a third and fourth animal were not included in this average because significant low-frequency fatigue had occurred in those experiments). Force enhancement was reduced after 20 ms of subsequent shortening ($+/-$ 1.0 L_0/s trials) whereas force depression was reduced after approximately 50 ms of subsequent lengthening ($-/+$ 1.0 L_0/s trials). A second axis of length change in units of nm per half-sarcomere is included. Because of the slow acceleration at the start of the length change, the first ~5 nm of length change occurred over a relatively longer time period. Extrapolation of the initial decay of force enhancement produced a zero at approximately 15 nm of shortening per half-sarcomere (thick gray line).

Table 1. Properties of force enhancement and force depression

	Transient properties			Residual properties	
	Force enhancement	Force depression	Shortening-induced de-activation	Force enhancement	Force depression
Duration of effect	Decays with time after stretch (less than 1–2 s)[a, b, c]	Decays with time after shortening (less than 1–2 s)[a]	Decays with time after shortening (less than 1–2 s)[d, e]	Does not decay with time (until activation ceases)[b, f, g, h]	Does not decay with time (until activation ceases)[i, j, k, g, h, l, m, n]
Dependence on filament overlap	Increases with length (see below)[a, c], but critical distances for recruitment and release are filament-overlap independent[c]	Greatest effect at ~1.0 L_0[a]	Greatest effect at 1.0 L_0[e]	Only on descending limb of FL relationship[b, f]	Increases with length[h, i, j, k, l]
Correlation with amplitude of length change	Positive only up to critical sarcomere extension[b, c]	(Unknown)	Positive[d]	Positive[b]	Positive[g, j, k, l, m, n]
Correlation with speed	None[c]	(Unknown)	None[d]	None[f, g, h]	Negative[g, i, j, k, l, n]
Correlation with load	None[a]	None[a]	None[d]	(Unknown)	Positive[g, i, j, k, l, n]
Correlation with work imposed/ done	None[a]	None[a]	None[d]	(Unknown)	Positive[k, m]
Correlation with activation level	None[a]	None[a]	Negative[e, o]	(Unknown)	Positive[n]
Change in force/ stiffnes ratio	(Unknown)	(Unknown)	(Unknown)	Decrease[g, h]	No change[g]
Shift in the FV relationship	No[b]	(Unknown)	(Unknown)	Yes[b]	Yes[k]
Shift in V_{max}	No[b]	(Unknown)	No[e]	No[b, p]	Perhaps[k]
Correlation with sarcomere non-uniformities.	(Unknown)	(Unknown)	(Unknown)	None[f, h] (But see below)	Positive[h, i] Partially, but not completely[k]
Effect of subsequent movement in opposite direction	Cancels force enhancement[a, c, e]	Cancels force depression[a]	None[e]	None[f]	(Unknown)

[a] Present series of experiments.
[b] Edman *et al.* (1978), intact frog fibers 0–3°C.
[c] Edman *et al.* (1981), intact frog fibers 0–3°C.
[d] Edman (1975), intact frog fibers 0–3°C.
[e] Edman (1980), intact frog fibers 0–3°C.
[f] Edman *et al.* (1982), intact frog fibers 0–3°C.
[g] Sugi and Tsuchiya (1988), intact frog fibers 0–3°C.
[h] Julian and Morgan (1979), intact frog fibers at 4–13°C.
[i] Edman *et al.* (1993), intact frog fibers 0–3°C.
[j] Herzog and Leonard (1997), feline whole-muscle at 37°C.
[k] Granzier and Pollack (1989), intact frog fibers 0–3°C.
[l] Maréchal and Plaghki (1979), whole frog muscles at 0–3°C.
[m] Herzog *et al.* (1998), feline whole-muscle at 37°C.
[n] De Ruiter *et al.* (1998), human whole-muscle *in vivo*.
[o] Ekelund and Edman (1982), skinned frog fibers at 0–3°C, skinned mouse fibers at 14–16°C.
[p] Edman and Tsuchiya (1996), intact frog fibers 0–4°C.

Edman *et al.* (1981) stated that they observed a maximal effect of transient force enhancement at ~1.15 L_0. However, if you correct their results for filament overlap effects, transient force enhancement increased with length up to the maximum length they tested (~1.45 L_0; their Figure 7B). Although no correlations were observed between sarcomere non-uniformities and residual force enhancement in the experiments cited above, Morgan (1994) suggested that the non-uniformities responsible for residual force enhancement may only be visible at the electron microscope level and not at the light microscopy level. Experimental work by Brown and Hill (1991) and Talbot and Morgan (1996) are consistent with this hypothesis.

study before entering their findings in a particular column. As can be seen in this table, we have included our results under the transient heading. Given the relatively short duration of our stimulus trains, we cannot be absolutely certain of the duration of our findings, but a number of other properties suggest that this classification is correct. The force enhancement that we observed was (i) cancelled by subsequent shortening and (ii) present at lengths shorter than L_0. Both of these properties are clearly associated with the previously

observed transient phenomena and are clearly not associated with the residual ones. The situation with force depression was different in that we observed it to be (i) independent of work done, (ii) independent of activation and (iii) reversed by subsequent stretch. The first two properties distinguish it from residual force enhancement and the latter two distinguish it from shortening induced de-activation. We felt it appropriate to suggest that what we observed was, in fact, a previously undescribed third component of force depression. Given the remarkable symmetry with transient force enhancement we have named it transient force depression.

Residual components

Although we did not observe the residual components of either force enhancement or force depression, it is necessary to understand these phenomena and their underlying mechanisms in order to separate their effects from transient phenomena described in other studies. Both Morgan (1994) and Edman *et al*. (1993) have independently provided strong evidence and arguments in favor of sarcomere non-uniformities as the cause for both residual force enhancement (Morgan) and residual force depression (Edman). They have each explained how this same hypothesis can explain nearly all of the properties listed in Table 1. However, it has been pointed out on at least two occasions (Sugi and Tsuchiya, 1988; Granzier and Pollack, 1989) that there are a few asymmetries in the properties of these two phenomena. This observation has then been used as 'evidence' against the possibility of a single mechanism for both phenomena. Because this issue was not addressed by either Morgan or Edman, we explain here why this conclusion is not necessarily correct.

There is a subtle but key difference in the effects of the non-uniformities proposed by Morgan and Edman. During shortening, Edman proposed that those non-uniformities in sarcomere strength that are inherently present in an isometric fiber will lead to a wider variability in sarcomere lengths during shortening, which will in turn lead to an even wider variability in sarcomere forces. During this process all sarcomeres remain active – i.e. they all produce active force. In contrast, Morgan proposed that these same non-uniformities in sarcomere strength would lead to an increase in sarcomere heterogeneity of a different sort during lengthening because of the negative slope of the FL relationship at longer lengths. This negative slope produces an unstable region of the FL relationship: sarcomeres that are lengthening within this region will become weaker as they get longer, leading to further lengthening which in turns leads to further weakening. Morgan proposed that when a lengthening sarcomere (or more properly a half-sarcomere) reaches this unstable region, it 'pops' to a much longer, more stable length beyond filament overlap. At these lengths the slope of the total FL relationship (including passive tension) becomes positive again and all force produced by that half-sarcomere is passive. The subtly different ways in which these two mechanisms produce residual force enhancement and depression account for the asymmetries listed in Table 2:

(i) *Stiffness asymmetries*. Because all force during residual force depression is active (i.e. produced by cross-bridges) one would expect the stiffness to change in proportion to force during residual force depression. In contrast, during residual force enhancement, part of the force is produced via passive elements, which have a much lower stiffness than cross-bridges, and thus one would expect stiffness to decrease relative to force.

(ii) *Difference between filament overlap dependence*. Sarcomere 'popping' during residual force enhancement is dependent upon the instability produced by the negative slope of the FL relationship. This instability only exists at lengths greater than L_0, so one would expect residual force enhancement only at lengths greater than L_0. In contrast force depression does not depend on such an instability and so there is no a priori reason to expect it to exist only at lengths greater than L_0.

(iii) *Difference in velocity dependence*. In both phenomena the inherent, small sarcomere non-uniformities in a fiber become amplified during motion because the FV relationship amplifies the force differences between sarcomeres. During shortening, force is heavily dependent upon velocity, and so the degree of amplification should be velocity dependent. In contrast, for much of the lengthening half of the FV relationship (i.e. at moderate to high speeds), force is independent of velocity and so the degree of amplification should be velocity independent. It is interesting to note that both Morgan (1994) and Edman *et al*. (1993) tested their hypotheses using computer simulations, and in both cases the computer simulations were able to confirm the validity of their hypotheses.

Transient force enhancement

As mentioned earlier, we believe that the transient force enhancement that we observed is the same phenomenon that has been examined previously by many researchers. To explain our findings, we start with the observation by Edman *et al*. (1981) that the critical distance for recruitment and release of transient force enhancement is independent of filament overlap. As argued by Noble (1992) it is difficult to imagine any non-cross-bridge-based hypothesis that could explain this finding. The simplest explanation for transient force enhancement arises straight from Huxley's original proposal for the mechanism of force production in muscle (Huxley, 1957) as later modified in Huxley and Simmons (1973). This hypothesis is reviewed here briefly.

In the cross-bridge model we assume that cross-bridges attach at or near some preferred angle with the thin filament θ_{att} (Figure 6A). As a muscle is stretched, the angles of all attached cross-bridges increase, skewing

Table 2. Theories of force enhancement and force depression

	Transient phenomena			Residual phenomena	
	Force enhancement	Force depression	Shortening-induced de-activation	Force enhancement	Force depression
Deactivation via decreased Ca affinity[a]	–	Incompatible – effect is activation independent[b]	Compatible	–	Incompatible – effect is not present when sarcomere length is controlled[c]
Deactivation via decreased Ca release[a]	–	Incompatible – effect is activation independent[b]	Incompatible – effect is present in skinned fibers[a]	–	Incompatible – effect is not present when sarcomere length is controlled[c]
Stress-induced cross-bridge inhibition[d]	–	Incompatible – effect is load independent[b]	Incompatible – effect is load independent[e]	–	Incompatible – effect is not present when sarcomere length is controlled[c]
Inhibition by increased metabolite concentration[f]	–	Incompatible – effect is activation independent[b]	Incompatible – effect is correlated negatively with activation[a, g]	–	Incompatible – effect is not present when sarcomere length is controlled[c]
Cross-bridge distribution re-settling[h]	Compatible	Compatible	Incompatible – effect is not reversed by subsequent stretch[g]	Incompatible – effect is residual[i, j]	Incompatible – effect is residual[c, d, f]
Sarcomere non-uniformities[c, k]	Incompatible – effect is transient[b, i, l]	Incompatible – effect is transient[b]	Incompatible – effect is transient[e, g]	Compatible	Compatible
Recruitment of a Passive element[g, j]	Incompatible – critical distances are filament-overlap independent[l]	Compatible	Incompatible – V_{max} is not affected by phenomenon[g]	Compatible	Incompatible – effect is not present when sarcomere length is controlled[c]

[a] Ekelund and Edman (1982), skinned frog fibers at 0–3°C, skinned mouse fibers at 14–16°C.
[b] Present series of experiments.
[c] Edman *et al.* (1993), intact frog fibers 0–3°C.
[d] Maréchal and Plaghki (1979), whole frog muscles at 0–3°C.
[e] Edman (1975), intact frog fibers 0–3°C.
[f] Granzier and Pollack (1989), intact frog fibers 0–3°C.
[g] Edman (1980), intact frog fibers 0–3°C.
[h] Huxley and Simmons (1973).
[i] Edman *et al.* (1978), intact frog fibers 0–3°C.
[j] Edman *et al.* (1982), intact frog fibers 0–3°C.
[k] Morgan (1994).
[l] Edman *et al.* (1981), intact frog fibers 0–3°C.

This table lists the major hypotheses that have been proposed over the years for the various phenomena. We then compared each hypothesis with the properties of each phenomenon listed in Table 1. Hypotheses that have been suggested only for force depression were not considered for force enhancement. In contrast, hypotheses put forward for either a transient or residual component were considered for both. Each hypothesis is labeled as Compatible with each phenomenon or Incompatible, in which case we list a single property with which the hypothesis is incompatible. This table seems to imply that for residual force enhancement there are two apparent hypotheses that are consistent with current data; as explained in the discussion, however, sarcomere non-uniformities are expected to lead to the 'recruitment' of passive elements. This table also points out that for transient force depression we cannot eliminate the possible recruitment of a passive element. As explained in the discussion, however, the parallels between transient force enhancement and transient force depression are remarkable, leading to the simplest hypothesis that they are caused by the same mechanism.

the distribution of attached cross-bridges to greater angles. As the angles increase, some of the cross-bridges will begin to detach, reattaching back at or near θ_{att} so that a steady-state distribution of attached cross-bridges will form, skewed to larger angles as shown in Figure 6A. Assuming that cross-bridge force increases with cross-bridge angle (Figure 6B; Julian and Sollins, 1975; Goldman *et al.*, 1988), changes in the distribution will be reflected in changes in the active contractile force produced by the muscle.

Transient force enhancement can be explained simply by changes in the distribution of attached cross-bridges with velocity and time. At the end of an active stretch, the distribution will, for an instant, be the same as it was during the stretch. Over time it will shift back towards the isometric distribution as attached cross-bridges detach and reattach at the preferred angle of attachment. A schematic of the time course of this effect is shown in Figure 6C. This effect will be observed macroscopically as a gradual decrease of force enhancement because the decreasing mean angle θ is associated with a smaller mean force per cross-bridge. The relative size of this effect should be dependent only on the distribution of attached cross-bridges and not on the

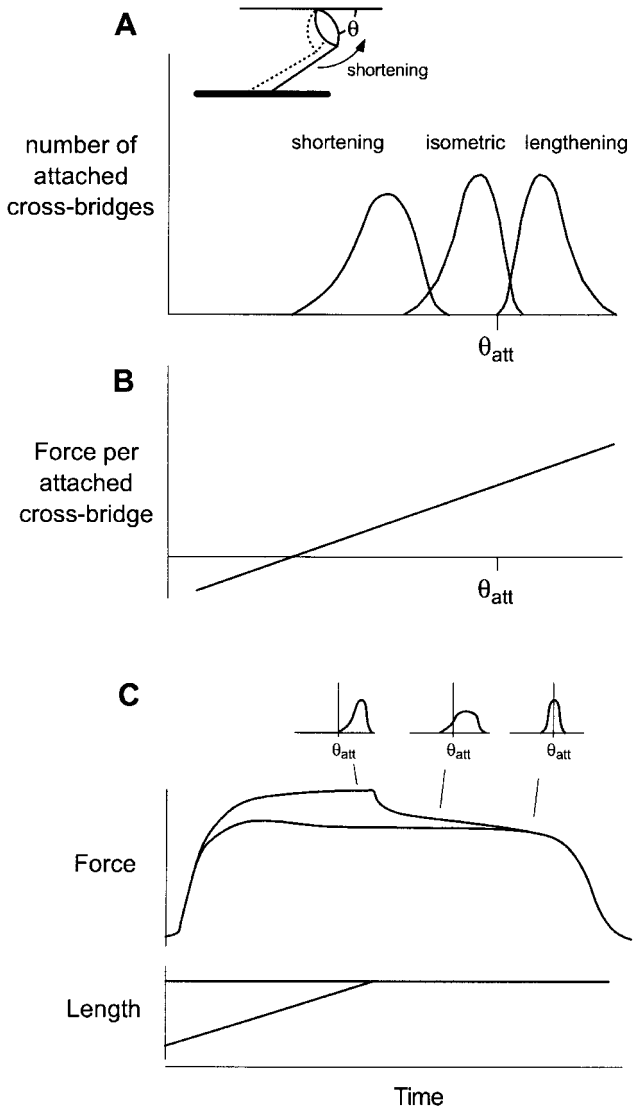

Fig. 6. Schematic description of cross-bridge model. (A) Qualitative description of the relative distributions of attached cross-bridges during steady-state shortening, isometric and lengthening conditions. Inset figure shows a schematic of the cross-bridge. As a muscle is allowed to shorten the myosin head rotates and the angle θ between the myosin head and the thin filament decreases. (B) Qualitative description of the relationship between cross-bridge force and θ. As θ increases so does cross-bridge force. (C) Inset figures show the hypothetical distributions of attached cross-bridges at various times during the decay of force enhancement. During lengthening the distribution skews to larger θ. The distribution shifts over time back to the isometric distribution as cross-bridges detach and reattach at θ_{att}. The effect of the skewed distribution shows up macroscopically as force enhancement because of the relationship between cross-bridge force and θ, shown in B.

fraction of attached cross-bridges. Our hypothesis is thus consistent with our results that the relative size of this effect was independent of work done on the muscle, load and activation.

Other evidence in support of this hypothesis was first presented by Edman *et al.* (1981). They showed that small length releases following a stretch could cancel the effects of transient force enhancement. The size of the release required to remove the force enhancement decreased over time in parallel with the decrease of force enhancement. These results are consistent with a slow shift over time of the distribution of attached cross-bridges from a stretched position back towards the isometric position (Figure 6C). Edman *et al.* also showed that the maximum size of the necessary release (~12 nm per half-sarcomere) was similar to the critical extension necessary to fully elicit force enhancement (~17 nm per half-sarcomere). These values are similar to the estimated 15 nm shortening per half-sarcomere required in our study to eliminate force enhancement (Figure 5C). More importantly, all of these particular findings were independent of sarcomere length as would be expected from the hypothesis.

Transient force depression

The transient force depression that we observed appears to be distinct from what has been called shortening induced de-activation (Edman, 1975, 1980). Although it is entirely possible that we have observed the same phenomenon and that differences in the preparations (e.g. temperature, whole-muscle vs. single-fiber) could explain the differences in our results, we believe that it would be difficult to suggest a single hypothesis that would be consistent with all findings. We therefore suggest that shortening induced de-activation, which has been characterized thoroughly by Edman in single fibers at cold, non-physiological temperatures, is different and independent of the transient force depression observed here in a whole-muscle at body temperature. It remains to be determined why we should see one phenomenon under certain conditions and the other one under different conditions.

Our hypothesis for transient force depression begins with the realization that if the hypothesis for transient force enhancement is correct, then one would expect a symmetrical effect following shortening. The transient force depression that we observed appears to be almost an exact mirror of transient force enhancement, suggesting that the underlying mechanisms are, indeed, similar.

Critical distances for transient components

With both transient force enhancement and transient force depression we expected that a subsequent length change in the opposite direction should reverse this effect. We first studied this hypothesis using slow length changes at $\pm 0.25\ L_0$/s following initial, transient force enhancement or depression with 200 ms length changes at $\pm 0.5\ L_0$/s (Figure 1C). Based upon previous findings (Edman *et al.*, 1981) we expected that shortening approximately 15 nm per half-sarcomere would cancel any initial force enhancement and that further shortening of some similar distance would create a force depression similar to that which would occur if the muscle had been shortening the whole time. Similarly, we expected that stretching some small distance would

cancel any force depression and that further stretching of 15 nm per half-sarcomere would create a force enhancement similar to that which would occur if the muscle had been stretched the whole time. To our surprise, after 40 nm of length change per half sarcomere there was still a large effect for both conditions (Figure 4B). Extrapolation suggested that the necessary length changes to cancel the differential effects of the initial movement would be approximately 100 nm. The more direct comparison of transient force enhancement and depression vs. the isometric condition presented in Figure 5 clarified these findings. Transient force enhancement was canceled by subsequent shortening of ~15 nm/half-sarcomere as expected, whereas transient force depression, for the conditions tested here, required subsequent lengthening of ~50 nm/half sarcomere to be canceled.

Because the physical stroke length for a cross-bridge is much less than 50 nm (Higuchi and Goldman, 1995), these results may at first seem contradictory to our hypothesis. However, Higuchi and Goldman (1995) and Kitamura *et al.* (1999) have shown recently that the working stroke length for a cross-bridge (shortening distance associated with the hydrolysis of a single ATP molecule) during high-speed shortening can be at least 30 nm and perhaps much larger. This finding implies that a single cross-bridge can interact with several actin binding sites during a single working stroke, temporarily detaching and re-attaching. The results shown in Figure 5B and 5C suggest, surprisingly, that this phenomenon is at least partially reversible – cross-bridges that have reached small angles θ due to shortening appear to be able to interact with more than one binding site during subsequent lengthening. If true, then this should produce an observable effect on slow-twitch muscle yielding if stretch were to be preceded by shortening. An interesting experiment that could be used to test our hypothesis would be to perform the inverse of Edman *et al.*'s (1981) study on force enhancement. Single fibers could be stretched virtually instantaneously after inducing transient force depression and the size of the stretch required to cancel the force depression noted. Our results seem to imply that large length changes, perhaps 50–100 nm, would be required to remove force depression. It is possible, however, that these interactions are velocity dependent and that very fast stretches may require significantly smaller length changes to cancel force depression.

These last results thus provide an explanation for the major problem associated with suggesting that transient force enhancement/depression can be explained by cross-bridge re-distribution. As pointed out by Noble (1992), the stress/strain relationship for cross-bridges based upon extremely fast length changes predicts that the critical distances necessary to produce force enhancement or release it should be on the order of 3–6 nm (Lombardi and Piazzesi, 1990). However, if cross-bridges can interact with multiple attachment sites at high speeds as suggested by Higuchi and Goldman (1995) and at low speeds as suggested here, then one of the major assumptions used to predict the size of these critical distances is no longer correct. One would have to quantify more carefully the attachment/re-attachment rates for intermediate cross-bridge states at various speeds to be able to predict these critical distances accurately.

Physiological relevance

If we are correct that the residual components of stretch-induced force enhancement and shortening-induced force depression were not observed in this study, then this suggests that the residual components and the proposed underlying mechanism of sarcomere non-uniformity may not exert much effect under physiological conditions. The residual components may be a feature of more extreme conditions such as large stretches during tetanic contractions; while useful for understanding mechanisms of muscular contraction, such conditions are not particularly physiological. What is perhaps more surprising is that we did not appear to observe shortening-induced de-activation. It remains to be seen if this absence is particular to feline caudofemoralis (and its 100% composition of fast-twitch muscle fibers) or if it is more generally a property of mammalian skeletal muscle operating at body temperature *in situ*.

Similar to the persistent components, the transient components of force depression and enhancement will probably exert relatively small effects during physiological muscle function. The effects of the transient components appear largest during isometric conditions or slow length changes following a relatively large length change. The effects are relatively smaller and last for much shorter durations during large changes in velocity (compare Figure 5C vs. Figure 4A). During normal behaviors, e.g. locomotion, changes in velocity tend to be relatively large (Goslow *et al.*, 1973), suggesting that these phenomena may not be significant for most operating conditions. Furthermore, these effects are most prominent when fascicle velocity changes rapidly. Sudden changes in whole-muscle velocity are not passed on to the fascicle when there is any significant amount of in-series compliance (Scott *et al.*, 1996). Most muscles have a large in-series compliance that will reduce the magnitude of force depression and enhancement *in vivo*. While these phenomena provide interesting clues about cross-bridge dynamics, they may exert negligible effects on force production by muscles during most natural motor activities.

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