Mysteries of Muscle Contraction

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According to the cross-bridge theory, the steady-state isometric force of a muscle is given by the amount of actin–myosin filament overlap. However, it has been known for more than half a century that steady-state forces depend crucially on contractile history. Here, we examine history-dependent steady-state force production in view of the cross-bridge theory, available experimental evidence, and existing explanations for this phenomenon. This is done on various structural levels, ranging from the intact muscle to the myofibrillar and isolated contractile protein level, so that advantages and limitations of the various preparations can be fully exploited and overcome. Based on experimental evidence, we conclude that steady-state force following active muscle stretching is enhanced, and this enhancement has a passive and an active component. The active component is associated with the cross-bridge kinetics, and the passive component is associated with a calcium-dependent increase in titin stiffness.

Keywords: sliding filament theory, cross-bridge theory, myofilaments, sarcomeres

Background

This article is based on the Borelli lecture presented at the American Society of Biomechanics conference in September of 2006, and it deals with work over the past 9 years on history-dependent changes in force production in skeletal muscles. Specifically, we will discuss the phenomenon of residual force enhancement following muscle stretching within the framework of the cross-bridge theory of muscle contraction. In order to set the stage, it is necessary to define what is meant by residual force enhancement and to review basic aspects of the cross-bridge theory.

Residual Force Enhancement. When a muscle is stretched while it is activated (also referred to as an eccentric contraction), and then kept at a constant length long enough for force transients caused by the stretch to disappear, the resulting isometric steady-state force will always be greater than the isometric force at that same muscle length (and same activation level) without prior stretch (Figure 1). This property of muscle has been termed residual force enhancement (Edman et al., 1982) and has been observed in human muscles, isolated muscle preparations, single fibers, and isolated myofibrils (Abbott & Aubert, 1952; De Ruiter et al., 2000; Edman et al., 1978, 1982; Herzog & Leonard, 2002; Lee & Herzog, 2002; Morgan et al., 2000; Sugi & Tsuchiya, 1988). Force enhancement is known to increase with increasing stretch magnitudes (e.g.,

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Abbott & Aubert, 1952; Herzog & Leonard, 2002), at least up to a certain threshold amplitude (Bullimore et al., in press), is long lasting (Herzog & Rassier, 2002), but does not seem to depend on the speed of stretching (Edman et al., 1982). At long lengths, it is associated with an increase in passive force (passive force enhancement; Herzog & Leonard, 2002, Figure 1), and force enhancement can be abolished instantaneously by deactivating the muscle (Abbott & Aubert, 1952; Herzog et al., 2003).

In the following, the phenomenon of force enhancement is studied on different structural levels, each of which has its advantages and limitations. For example, demonstrating that there is force enhancement during voluntary contractions in intact human muscles makes this property important from a functional point of view, and studying this property on the myofibrillar level and in isolated contractile filaments allows for the determination of the molecular mechanisms that are responsible for force enhancement. In the following, we will introduce the cross-bridge theory and its basic properties. Most importantly in the present context is the fact that the cross-bridge theory cannot account for force enhancement without major changes in some of its basic underlying assumptions (Huxley, 1957). Therefore, studying force enhancement is of value to learn more about the functional properties of muscles, on the one side, and the molecular mechanisms of contraction, on the other side.

**The Cross-Bridge Theory.** The cross-bridge theory of muscle contraction states that shortening of a muscle and force production occur through the interaction of the two contractile proteins actin and myosin. Myosin has protrusions (cross-bridges) that cyclically attach to actin and draw the actin toward the myosin filament, thereby creating force and shortening. Each cross-bridge attachment/detachment cycle is powered by the hydrolysis of one molecule of adenosinetriphosphate (ATP). The cross-bridge theory was first formulated in mathematical terms by Huxley (1957) for two states, one attached and one detached state of the cross-bridge. Since 1957, cross-bridge models have become more sophisticated, in that more than two states corresponding to mechanical and or ATP hydrolysis events have been considered. However, the basic properties of the original cross-bridge model have been retained; that is, a number of cross-bridge states are connected by appropriate rate constants for the forward and backward reactions from one state to the next (Figure 2).

When solving the simplest of these cross-bridge models, as Huxley did in 1957, it becomes apparent that the steady-state force of a muscle is simply a function of the constants that govern the cross-bridge attachment and detachment rates (Equation 1).

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    n_{eq} = \frac{f(x)}{f(x) + g(x)}
\]

where \( n_{eq} \) is the proportion of attached cross-bridges at equilibrium or steady state, and \( f \) and \( g \) are the rate constants of cross-bridge attachment and detachment, respectively, which are functions of \( "x" \), the distance from the cross-bridge equilibrium position to the nearest actin attachment site. Note that in Equation (1) it is assumed that all cross-bridges have an identical \( "x" \)-value, in accordance with Huxley (1957). For a general solution of the cross-bridge theory, see Epstein and Herzog (1998).
One of the characteristics of these rate constants throughout history has been that they merely depend on the distance of the equilibrium position of a cross-bridge to its nearest attachment site (Figure 3). This distance has been referred to as Huxley’s $x$-distance, and it is this definition that makes Equation (1) independent of the history of contraction. In other words, the cross-bridge theory does not allow for steady-state isometric forces to differ, except if muscle activation or length is different.

Therefore, the cross-bridge theory does not contain an explanation, or a provision, for the residual force enhancement, but residual force enhancement had been observed systematically before the conception of the cross-bridge theory (e.g., Abbott & Aubert, 1952). Thus, force enhancement is an intriguing phenomenon, first because it is observed consistently, but has no accepted explanation, and second because it does not fit into the basic paradigm of the cross-bridge theory of muscle contraction. Therefore, study of force enhancement might provide new and unique insights into the mechanisms of contraction.

### The Sarcomere Length Nonuniformity Theory

For a naïve observer, it might seem strange that findings on force enhancement have been accumulated for over half a century, but few if any attempts have been made to explain this phenomenon within the
framework of the cross-bridge theory. The primary reason for this state of affairs is the fact that force enhancement has been explained traditionally with a theory that did not require changes to the existing cross-bridge thinking. It was based on the idea that during active stretching of a muscle, sarcomere lengths would become nonuniform and some sarcomeres would be stretched by only a tiny amount (if at all), whereas others, so-called weak sarcomeres, would be pulled beyond actin–myosin overlap and lose their ability to produce force, and would be held merely by passive structural elements (Figure 4). This theory was termed the sarcomere length nonuniformity theory and was based on the notion that sarcomeres are unstable on the descending limb of the force–length relationship (e.g., Hill, 1953). Two of the more important predictions of the sarcomere length nonuniformity theory are that force enhancement cannot occur on the ascending limb of the force–length relationship (because that part of the relationship has a positive slope and, therefore, does not allow for the presumed sarcomere length instabilities) and that steady-state force in the enhanced state cannot exceed the purely isometric reference forces on the plateau of the force–length relationship (because, independent of the nonuniformities in sarcomere lengths, forces in the enhanced state are given by the strongest sarcomeres and they can reside at no better place in terms of force production than the plateau of the force–length relationship; Figure 4).

**Figure 3** — Schematic representation of the classic cross-bridge model (Huxley, 1957; with permission) with the associated rate constants for attachment (f) and detachment (g). The rate constants merely depend on the distance between the cross-bridge equilibrium position and the nearest cross-bridge attachment site on actin (x-distance). Note the asymmetric arrangement of the attachment and detachment rate constants, which ensures that cross-bridges can exert force in one direction only.

**Figure 4** — Schematic representation of force enhancement according to the sarcomere length nonuniformity theory. Imagine an isometric reference contraction with uniform sarcomere length at two locations on the descending limb of the force–length relationship (open circle and filled square); the corresponding forces are given by the sliding filament theory and they lie on a straight line between a sarcomere length of 2.2 µm (100% force) and 3.6 µm (0% force). Now imagine that the muscle is stretched from the short sarcomere length to the long sarcomere length while active. According to the sarcomere length nonuniformity theory, some sarcomeres will only be stretched by a small amount (filled circle left) while others (weak sarcomeres) are pulled beyond myofilament overlap and are held only by their passive forces (filled circle right). Force equilibrium between these nonuniform sarcomeres is achieved when the passive force of the long sarcomeres is identical to the force of the short sarcomeres (horizontal dashed line). This force is then higher than the expected isometric force for uniform sarcomeres (= force enhancement, FE), as the short sarcomeres are stronger than the sarcomeres at the average length.
Testing of Predictions

When starting research on residual force enhancement, the first experiments were aimed at testing the two major predictions arising from the sarcomere length nonuniformity theory. The reason for this approach was quite simple: The sarcomere length nonuniformity theory was not appealing intuitively for a variety of reasons. First, it appeared odd that the molecular motor for muscle contraction would be unstable on the descending limb of the force–length relationship; after all, this portion covers more than half of the working range of muscle contraction (e.g., Gordon et al., 1966). Furthermore, if the descending limb was unstable, only a single sarcomere could reside on it in a fixed-end contraction (Allinger et al., 1996; Zahalak, 1997); all other sarcomeres would have to go onto regions of stability, either on the ascending or the passive limb of the force–length relationship. Finally, the descending limb of the force–length relationship was assumed unstable because of its negative slope. However, a negative slope obtained from isolated static tests (i.e., a muscle is activated at a length, and isometric force is measured; the muscle is then deactivated, set to a new length, and then activated again) does not say anything about the stability of the system. Specifically, it has been shown that when a muscle is stretched, its force will always increase, independent of its length, so the predicted negative force–length slope is caused by the static nature of obtaining the force–length property; it is not an inherent dynamic property of muscle.

Isolated Muscle Preparation. The first tests on force enhancement in our lab were made on intact, isolated cat soleus preparations. The muscle and its nerve were isolated, the distal end of the soleus tendon was attached to a muscle puller for length changes and force measurement, stimulation was performed via a nerve cuff electrode, and a variety of stretch experiments and the corresponding isometric reference contractions were performed in a total of approximately 35 muscles to date. For details of the methods, please be referred to the original papers (Herzog & Leonard, 2000, 2002, 2005; Schachar et al., 2002; Herzog et al., 2003). All tests on cat soleus were performed at physiologically relevant temperatures of 35–37 °C.

The major findings of these experiments were that force enhancement was observed on the ascending limb of the force length–relationship (Figure 5), and that force in the enhanced state could easily exceed the isometric reference forces obtained on the plateau of the force–length relationship (Figure 6). At a first glance, one could argue that...
two of the major predictions of the sarcomere length nonuniformity theory were violated; therefore, this theory should be rejected. However, measurements on a whole muscle preparation have the disadvantage that fiber lengths are known to be nonuniform. Therefore, one might argue that although it appears that the muscle was operating on the ascending limb of the force–length relationship, maybe that was only true for 80% of the fibers. The remaining 20% might have been on the descending limb of the force–length relationship and might have produced the observed force enhancement while the majority of the fibers gave the appearance of ascending limb behavior for the whole muscle. This limitation could not be ignored. Therefore, we decided that future experiments needed to be made on single-fiber preparations, thereby eliminating the possibility of fiber length nonuniformities affecting the outcome of force enhancement tests.

However, before describing the single-fiber experiments, three additional results that were observed in the cat soleus preparation deserve attention. First and probably most important, when stretching soleus muscles at long lengths corresponding to the descending limb of the force–length relationship, we observed that the passive force after deactivation was always greater than the passive force obtained when the muscle was activated isometrically at the same length or if the muscle was stretched passively (Figure 1). This observation was termed passive force enhancement, and it was shown that this passive force enhancement contributed to the total force enhancement (Herzog & Leonard, 2002). However, because the passive force enhancement was always smaller than the total force enhancement and because it did not occur at short lengths (where residual force enhancement was observed), it was hypothesized that the total force enhancement had at least two components—one corresponding to the passive force enhancement and the other corresponding to the remnant part of the residual force enhancement that could not be explained with the passive part. Furthermore, it was observed that force enhancement in the cat soleus was associated with a small but statistically significant increase in stiffness and with a decrease in the rate of force decay upon muscle deactivation (Herzog & Leonard, 2000).

**Single-Fiber Preparation.** Single fibers were isolated from the tibialis anterior and the lumbral muscles of frogs (*Rana pipiens*). Fibers were attached to a muscle puller at one end, and a force transducer at the other end in an experimental chamber whose fluid and temperature could be controlled. Experiments were performed at 8–10 °C and stimulation of the fibers was accomplished via two platinum wire electrodes that ran along the fiber on either side. For details of the single-fiber preparation, please refer to the original papers (Rassier et al., 2003; Peterson et al., 2004; Lee et al., 2007).

As for the whole-muscle preparation, force enhancement in single fibers was found on the ascending limb of the force–length relationship (Peterson et al., 2004) (Figure 7), and force in the enhanced state was shown to exceed the isometric reference forces on the plateau of the force–length relationship (Rassier et al., 2003; Peterson et al., 2004; Lee et al., 2007) (Figure 8). Also, all remaining observations made in the cat soleus (passive force enhancement, increased stiffness, and decreased rate of force decay in the enhanced compared with the isometric reference state) were also made in the single-fiber preparations.

We concluded from these results that the sarcomere length nonuniformity theory had become
untenable, and that it seemed likely that force enhancement was caused by an active and a passive component. However, the single-fiber preparation also had its limitations. For example, despite measuring average sarcomere length using laser diffraction (ter Keurs et al., 1978), it is impossible to know individual sarcomere lengths in this preparation, and it is not clear whether individual sarcomeres ever reached a steady-state length following fiber stretching, or whether sarcomeres were still shortening and lengthening as the force enhancement measurements were made.

In order to address this issue, we decided that measurements on the single myofibril level were required. Single myofibrils have the advantage over other preparations that individual sarcomere lengths can be measured continuously (Bartoo et al., 1993) and that all sarcomeres are arranged in series; thus, force recordings represent the force sustained by each sarcomere. Therefore, the purpose of the myofibril experiments was to test whether there was force enhancement in this preparation and whether it was associated with constant sarcomere lengths. Furthermore, the myofibril preparation would allow for absolute determination of the predictions of the sarcomere length nonuniformity theory because it would be possible to see whether sarcomeres were uniform in the isometric state and would become nonuniform with stretch, and whether sarcomere lengths were unstable on the descending limb of the force–length relationship as predicted by Hill (1953) and Julian and Morgan (1979).

**Isolated Myofibril Preparations.** Single myofibrils were isolated from rabbit psoas using published protocols (Bartoo et al., 1993; Blyakhman et al., 2001). They were attached at one end to a rigid glass needle, which in turn was attached to a motor that could produce length changes with an accuracy of better than 1 nm. The other end of the myofibril was attached to one nanolever of a pair whose stiffness was known (Figure 9). Upon activation and force production, the nanolever would move away from its twin, and, by measuring the deflection, forces could be determined. Individual sarcomere lengths were obtained by capturing the dark–light striation patterns of the myofibrils corresponding to the A-band and Z-lines (dark) and the I-bands (light) on a high-resolution (6-nm) linear photodiode array consisting of 10,680 elements (Figure 10). Activation of the myofibrils was achieved chemically by

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**Figure 8** — a) Force–time and the corresponding sarcomere length–time histories for an isometric reference contraction at the final length (f) and the optimal length (o), and a stretch-test contraction (s) of a single fiber from the lumbrical muscle of the frog, *Rana pipiens*. Note that the steady-state force following stretch is greater than the force at the final stretch length (= force enhancement, FE) and is greater also than the isometric force at optimal fiber length (force above plateau, FP). b) Force–length relationship of frog single fibers and the corresponding forces following stretches of various magnitudes on the ascending and descending limb of the force–length relationship. Note the force enhancement following active stretches and that force following active stretching can easily exceed the maximal isometric forces obtained at the plateau of the force–length relationship.

**Figure 9** — Myofibril attached to a rigid needle at one end (which in turn is connected to a motor for computer-controlled length changes of the myofibril) and to a pair of nanolevers for force measurement at the other end.
Sarcomere lengths on isolated myofibrils were nonuniform before and after active stretch but were perfectly stable for the isometric contractions preceding and following stretch (Figure 11). Furthermore, each myofibril tested \( (n = 12) \) showed force enhancement, and each sarcomere of all myofibrils \( (n = 78) \) showed force enhancement following active stretch (Figure 12). Eleven of the twelve myofibrils also showed forces in the enhanced state that exceeded (sometimes by more than 100%) the isometric forces at optimal sarcomere length; here, optimal length was defined when the average sarcomere length of a myofibril corresponded to optimal overlap between actin and myosin filaments, that is, 2.26–2.43 \( \mu \text{m} \) (Page & Huxley, 1963; Herzog et al., 1992). Finally, isolated myofibrils also showed a significant amount of passive force enhancement when stretched from an average sarcomere length of 2.4 \( \mu \text{m} \) to 3.4 \( \mu \text{m} \) (Figure 13).

From these results, the following conclusions were drawn. Force enhancement and passive force enhancement exist on the level of single myofibrils.
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and single sarcomeres; force enhancement is achieved with sarcomere lengths perfectly constant (stable) on the descending limb of the force–length relationship; and sarcomere lengths are nonuniform in purely isometric reference contractions and in isometric contractions preceding and following active myofibril stretching. Therefore, it seems that sarcomere length nonuniformity is not the cause for the increased forces following stretch; rather, there is an active and a passive component of force enhancement on the myofibril and sarcomere level.

Molecular Mechanisms of Force Enhancement

In order to elucidate the molecular mechanisms for the force enhancement observed on all structural levels presented here (Lee & Herzog, 2002) and others that were not presented (for example, voluntary contractions in human skeletal muscles, which also show force enhancement; Oskouei & Herzog, 2005), it was necessary to formulate precise hypotheses about the active and the passive component of force enhancement.

Active Component of Force Enhancement. For the active component, we hypothesized that force enhancement was associated with an increase in the proportion of attached cross-bridges following stretch, compared with the proportion of attached cross-bridges during a purely isometric reference contraction. An increase in the proportion of attached cross-bridges could be achieved with either an increased rate of cross-bridge attachment or a decreased rate of detachment. Based on work with other (nonmuscle) myosin motors (Veigel et al., 2005; Altman et al., 2004; Clemen et al., 2005; Veigel et al., 2003), it was speculated that the rate of detachment was decreased and thereby the time of attachment (dwell time) was increased, and the time a cross-bridge was attached relative to the whole cross-bridge cycle time (the duty ratio) would also be increased following stretch.

In order to test this hypothesis, we had to find a preparation in which we could stretch a single attached cross-bridge. This was accomplished by isolating single actin filaments and having them interact with single cross-bridges in a laser trap setup similar to the one described first by Finer et al. (1994). In this setup, two micron-sized beads...
are attached to the ends of an actin filament and the beads are held with two independently controlled laser beams. This laser trap setup allowed for manipulation of the actin filament in any desired way. Specifically, the actin filament was brought into the vicinity of an isolated cross-bridge (a heavy meromyosin construct) that was placed on top of a bead on the coverslip of an inverted microscope (Figure 14). Attachment of the cross-bridge was identified by a reduction in the Brownian noise of the actin beads, and the cross-bridge was then stretched or shortened while attached to the actin filament (Figure 15). The corresponding dwell time and duty ratios were measured and compared.

Measurements were made on seven constructs (actin and cross-bridge heads) for over 900 stretching and shortening experiments. The dwell times and duty ratios for the two conditions were 255 ms (±236 ms) and 8.3% (±3.0%) for the stretching and 230 ms (±200 ms) and 7.3% (±2.1%) for the shortening conditions. They were statistically the same, thereby rejecting the hypothesis that stretching of attached cross-bridges causes an increase in dwell time and duty ratio compared with shortening. There are a variety of limitations associated with the tests as performed here. Most importantly, all actin–myosin interactions were performed at a low-ATP concentration (≤0.1 µM), thereby creating a rigorlike environment that might have given results that are not representative of the actual cross-bridge kinetics at physiological levels of ATP (about 8 mM). Therefore, although the results did not support the proposed hypothesis, they must be considered with some caution. Performing the experiments at physiological ATP concentrations might be impossible at present because of the fast ADP release rate under these conditions (<1 ms) and the associated difficulties in resolving this step with the laser trap approach.

### Passive Component of Force Enhancement

For the passive component of force enhancement, we hypothesized that it was caused by a calcium-dependent increase in the stiffness of titin. Titin is a structural protein spanning half sarcomeres and making attachments in the center and end of the sarcomere at the M-line and the Z-line, respectively (Figure 16). Titin is prevalent in skeletal muscle and is thought to occur in a ratio of 6 to 1 with myosin. In single myofibrils, titin is typically the primary source for passive force. Therefore, we wanted to test the hypothesis using single-myofibril preparations. The idea was to stretch myofibrils at low and high calcium concentrations and measuring the passive forces. If our hypothesis was correct, then (a) there should be passive force enhancement with calcium activation of myofibrils and (b) the passive force enhancement would be caused exclusively by an increase in titin stiffness.

In order to test whether there was passive force enhancement, six myofibrils were stretched from an average sarcomere length of 2.4 to 3.4 µm, first passively and then actively. These experiments confirmed that there was passive force enhancement...
in this preparation (Figure 13), which averaged 39 nN/µm². Now, the experiment had to be repeated with a low and high calcium concentration, but in order to see any possible calcium dependence of the passive force, active forces needed to be abolished. This was achieved by chemically stripping troponin C (a regulatory protein responsible for the control of cross-bridge attachments) from actin, thereby inhibiting all active forces (Figure 17). The troponin C–stripped myofibrils were now stretched at calcium concentrations of pCa[8.5] and pCa[4.5], and it was found that in the absence of any active force, there was passive force enhancement in these constructs (Figure 18), thereby confirming that calcium activation was responsible for increasing passive forces and therefore contributing to the passive force enhancement. Since most of the passive force in rabbit psoas myofibrils (used here) is caused by titin, it would be safe to assume that the increase in

Figure 16 — Schematic illustration of titin’s location within a half sarcomere. Also indicated are the supposed Ca binding sites on titin (square symbols on titin) and possible sites for titin attachment to actin (round symbols on titin). Note that the Ca binding sites and the actin attachment sites illustrated here are schematic and do not correspond to the actual location of these sites. When a sarcomere is stretched passively (top two panels), there is a passive force, which in rabbit psoas sarcomeres is almost exclusively caused by the molecular spring titin. When a sarcomere is stretched actively, but active force is inhibited by troponin C depletion (third panel from top), “passive” force is greater than for the purely passive stretch (second panel from top), possibly because of attachment of calcium ions (from the activating solution) to specific calcium binding sites on titin (third panel from top; filled squares, labeled Ca). Finally, when an intact (troponin C was not deleted) sarcomere is stretched while activated, the passive force is greatest (bottom panel) presumably because of calcium attachment to titin (as in the third panel) plus calcium-regulated titin attachment to actin, which shortens the resting length of titin, and thus provides further rigidity.

Figure 17 — Force–time histories for activated myofibrils at average sarcomere lengths of 2.4 and 3.4 µm before and after troponin C depletion. Note that forces after troponin C depletion are zero (triangles). Therefore, a troponin C–depleted myofibril can be activated with calcium without producing contractile forces, thereby allowing for measurement of the effect of Ca activation on passive structures of myofibrils.

Figure 18 — Force–time history of a troponin C–depleted myofibril while undergoing stretch at a low (pCa[8.5]; circles) and a high (pCa[4.5]; triangles) calcium concentration. In the troponin C–depleted myofibril, there is no active contractile force, thus indicating that the greater forces at the high calcium concentration are associated with a Ca-induced increase in titin stiffness.
passive force in the troponin C–depleted myofibrils was associated with an increase in titin stiffness. However, the passive force enhancement on the troponin C–depleted constructs averaged only 9 nN/µm², which is less than 25% of the total passive force enhancement observed in the intact constructs. Therefore, we concluded from these experiments that titin’s stiffness is likely regulated by calcium activation but that the increase in titin stiffness could not explain the entire passive force enhancement.

Conclusion
From the results of our combined work, the following conclusions seem warranted.

1. Force enhancement comprises at least two underlying components; we called them tentatively the passive component and the active component of force enhancement to indicate their likely origin.

2. The passive component of force enhancement is caused in part by a calcium-dependent increase in passive force, which is likely associated with an increase in stiffness of the molecular spring titin. We speculate that the rest of the passive force enhancement is related to a calcium, or troponin C, or force-dependent regulation of titin attachment to actin (Figure 16).

3. The origin of the active component of force enhancement remains unknown, but does not seem to be caused by a stretch-dependent increase in the dwell time or duty ratio of cross-bridges, although this needs further verification in experiments performed at physiological ATP levels.

4. The sarcomere length nonuniformity theory, based on unstable sarcomere lengths on the descending limb of the force–length relationship, did not receive support in any of our studies at the various structural levels. First, and contrary to the predictions of the sarcomere length nonuniformity theory, we observed force enhancement on the ascending limb of the force–length relationship, and observed forces in the enhanced state that were substantially greater than the maximal isometric forces at optimal muscle (fiber, sarcomere) lengths. Furthermore, sarcomeres in single myofibrils were perfectly stable on the descending limb of the force–length relationship, and sarcomere length nonuniformity existed in purely isometric contractions and not only following muscle stretch.

5. Sarcomeres at distinctly different lengths on the descending limb of the force–length relationship, and thus different amounts of actin–myosin overlap, produced the same force. This observation could not be explained with differences in passive forces, differences in half-sarcomere kinetics, or differences in the amount of contractile proteins across sarcomeres. Therefore, we conclude that myofilament overlap alone cannot account for the maximal isometric steady-state force a sarcomere can exert.

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