Force enhancement after stretch in mammalian muscle fiber: no evidence of cross-bridge involvement

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Nocella M, Cecchi G, Bagni MA, Colombini B. Force enhancement after stretch in mammalian muscle fiber: no evidence of cross-bridge involvement. Am J Physiol Cell Physiol 307: C1123–C1129, 2014—Stretching of activated skeletal muscles induces a force increase above the isometric level persisting after stretch, known as residual force enhancement (RFE). RFE has been extensively studied; nevertheless, its mechanism remains debated. Unlike previous RFE studies, here we report the first evidence of force enhancement after stretch, termed static tension (ST), in isolated individual golden hamster flexor digitorum brevis (FDB) mouse muscle at 30°C. ST was measured as the difference between the maximum tetanic tension developed at the stretched length and the tension attained during an isometric hold phase of the stretch, the force settles down to a steady isometric force. At the end of the force transient, during the fiber stretch, a transient increase of tetanic force well above the isometric force (29). This unexpected result prompted us to investigate the possibility that ST in intact fibers from mouse muscle also could show a similar dependence from sarcomere length.

Materials and Methods

Fiber dissection and mounting. This study was carried out following the EEC guidelines for animal care of The European Community Council (Directive 86/609/EEC), and the protocol was approved by Italian Health Ministry and the Ethical Committee for Animal Experiments of the University of Florence (acceptance signed by the veterinary responsible in October 20, 2010).

C57BL/6 male mice were housed at controlled temperature (21–24°C) with a 12:12-h light-dark cycle, and food and water were provided ad libitum. Mice (4–5-mo-old) were killed by rapid cervical dislocation. Small intact fiber bundles (10–20 fibers) from the flexor digitorum brevis (FDB) muscles of the hindlimb of both legs were dissected manually under a stereomicroscope with a pair of fine scissors and needles. Particular care was taken to avoid excessive dislocation. Small intact fiber bundles (10–20 fibers) from the flexor digitorum brevis (FDB) muscles of the hindlimb of both legs were dissected manually under a stereomicroscope with a pair of fine scissors and needles. Particular care was taken to avoid excessive dislocation. Small intact fiber bundles (10–20 fibers) from the flexor digitorum brevis (FDB) muscles of the hindlimb of both legs were dissected manually under a stereomicroscope with a pair of fine scissors and needles. Particular care was taken to avoid excessive dislocation. Small intact fiber bundles (10–20 fibers) from the flexor digitorum brevis (FDB) muscles of the hindlimb of both legs were disse...
stretching and to obtain bundles as clean as possible from connective tissue and debris from dead fibers. The number of animals used was minimized by using both leg muscle and by dissecting more than one bundle from the same muscle. Bundles were mounted horizontally, by means of aluminum foil microclips compressed onto the tendons, between the lever arms of a capacitance force transducer (resonance frequency: 25–50 kHz) and a fast electromagnetic motor (minimum stretch time: 100 μs), which could apply to the bundles stretches of the desired shape and amplitude. The experimental chamber (capacity: 0.38 ml) was provided with a glass floor for light illumination. The motor was mounted on a micromanipulator to adjust the passive length of the bundles at selected values. Bundles were perfused permanently by means of a peristaltic pump at a rate of ~0.35 ml/min with a normal Tyrode (NT) of the following composition (mM): 121 NaCl, 5 KCl, 1.8 CaCl2, 0.4 NaH2PO4, 24 NaHCO3, 5.5 glucose, and 0.1 EDTA. This solution was continuously bubbled with a mixture of 5% CO2-95% O2, which gave a pH of 7.4. Fetal calf serum (0.2%) was freshly added to the solution. In a group of experiments 10 μM BTS were added to the NT solution to reduce active tension and cross-bridge influence on the response to the stretches. Stimuli of alternate polarity, 0.2- to 0.5-ms duration and 1.5 times threshold strength, were applied transversely to the bundles by means of two platinum-plate electrodes running parallel to them. After a test of viability, the desired sarcomere length was set by adjusting appropriately the bundle length with the micromanipulator. Clip to clip fiber length including tendon (l0), end to end without tendons (l0), and sarcomere length were measured using a microscope fitted with a ×20 eyepieces and a ×5 or ×40 dry objective in the experimental chamber and on digital images acquired by a videocamera (Infinity Camera, Lumenera, Canada) assisted by an image processing software. The cross-sectional area was calculated as if the section of the bundles were elliptical, with the formula \(a b\), where \(a\) and \(b\) are the smaller and the greater diameters measured along the bundles. Resting sarcomere length was measured by counting the number of consecutive sarcomeres in a calibrated scale on the pictures taken with the videocamera. After 20 min of equilibration, tetanic stimulation was applied in brief (300-ms duration) volleys using the minimum frequency necessary to obtain a fused contraction (~100 Hz). In general, plateau tetanic force (\(P_{\text{th}}\)) was stable over a period of few hours. A custom-written software (LabView; National Instruments) was used to drive the stimulator and the electromagnetic motor and to record force and fiber length at two different sampling times of 1 ms and 10 μs to obtain the appropriate time resolution for all the parts of record.

**ST measurements.** ST was measured as described previously (2, 3, 6). Briefly, three force records were taken for each measure: 1) the response of the active fibers to fast stretches and hold; 2) the response of the passive fibers to the same stretch; and 3) the isometric response. By subtracting the passive and the isometric responses from the response to fast stretch, we obtained the excess of force induced by the stretch. After a peak, synchronous with the stretch, the excess of force settled within few milliseconds to a steady value that represents the ST value. Stretches used were ramp shaped with an amplitude of 3–4% \(l_0\) and a stretch time of 0.6–0.7 ms, corresponding to a stretching velocity \(\geq 70 l_0\) s\(^{-1}\), and were applied at low tension on the rise of a short tetanus (3 stimuli). The use of fast stretches and their application at low tension reduced to <10 ms the time necessary for the tension to settle to the steady level after the stretch, much less than the \(~2\) s required when slow stretches were applied at tetanus plateau as in previous RFE studies (10, 11, 29).

Data values are expressed as means ± SE.

**RESULTS**

The experiments presented in this article were made on a total of 10 fiber bundles. Mean \(l_0\) and \(l_{\text{th}}\) were 1,161 ± 36 and 627 ± 12 μm, respectively. The fraction of stretch applied at the sarcomere level was calculated by correcting for the fraction absorbed by the tendons using the average ratio between tendon and fiber compliances reported previously (34, 36). This ratio was relatively independent of tension; therefore, it was assumed for all bundles that tendon compliance was 41% of total compliance whereas the remaining 59% was attributed to the fiber independently of the tension at which the stretch was applied.

**Static tension.** Figure 1 shows an example of force response to a fast stretch. Traces \(b\) and \(c\) are the force responses with and without the stretch, respectively. It can be seen that the tension after the stretch runs parallel but well above the isometric trace \(c\). The subtraction of the two traces, corrected for the passive response to the stretch (trace \(d\)), is shown by the trace \(e\) and represents the excess of tension above isometric induced by the stretch. After the end of the initial transient, due mainly to the cross-bridge response to the stretch, the tension settles to an approximately constant value, the ST, that lasts for the whole record. ST is generated during the stretch (see Fig. 2), but the presence of the initial force transient does not allow its measurements immediately after the stretch itself. However, given the steady force response, its value can be read without significant error some milliseconds after the stretch, as we did. The observation that ST remains almost constant for all the stimulation period, in spite of active tension changes, indicates that activation increases the parallel stiffness of the sarcomeres without inducing any change in their length. Thus sarcomere stiffening does affect force and can be detected only by stretching as we did. If sarcomere stiffness increases further after the stretch, again no changes will occur on force and a new stretch would be needed to detect it. Similar records to that of Fig. 1 were obtained with stretches applied at various delays from the start of stimulation. Figure 2 shows the force response

![Image](https://via.placeholder.com/150)

Fig. 1. Stretch applied on the tetanus rise induces a steady force potentiation. Fiber force response to a stretch of 3.1% fiber length without tendon (\(l_0\)) amplitude and 0.63-ms duration applied 3 ms after the stimulus when isometric tension was 1.65% plateau tetanic force (\(P_{\text{th}}\)) on the rise of a short tetanus (3 stimuli); trace \(a\): fiber length change; trace \(b\): isometric force with stretch; trace \(c\): isometric force; trace \(d\): passive response to the stretch. Trace \(e\) is obtained by subtracting traces \(c\) and \(d\) from \(b\) and represents the excess of tension induced by the stretch. The static tension (ST) is measured after the end of the fast transient, as indicated by the arrow. Sarcomere length = 2.65 μm. Sampling time is 10 μs between the dashed lines and 1 ms outside the lines. Horizontal dashed line indicates zero tension.
to a stretch applied 1 ms after the stimulus, during the latent period when active force is zero, compared with the force response at the 3-ms delay shown in Fig. 1.

The figure shows some interesting features: 1) although smaller, the steady tension after the stretch is present also at a 1-ms delay when active force is zero; 2) the initial peak of the force transient and the slow decay to the steady tension present on the record at a 3-ms delay have almost disappeared in the record at 1 ms, due to the absence of attached cross bridges; and 3) the steady tension after the stretch is established with no delay during the stretch itself. All these features show the independency of the ST from the active tension developed by the fibers.

The observation that ST is established during the stretch itself and that it remains constant after the stretch allowed us to measure the time course of ST development after the stimulus. To do this, stretches were applied at various delays from the start of stimulation in a series of short tetani (6, 35). A typical time course of ST development is compared with active tension time course in Fig. 3.

Stretch were applied between 0 and 16 ms after the start of stimulation. The figure shows, in agreement with previous reports (2, 4), that the development of ST precedes tension development being similar to the internal Ca²⁺ time course (5). No correlation exists between ST and active tension developed by the fibers at the time of the stretch. Note, for example, that ST has about the same maximum value at ~3-ms and ~12-ms delay, whereas the active tension at 12 ms is more than five times greater than at a 3-ms delay. The two peaks on the ST record occur at a 2- to 3-ms delay from the first and the second stimulus of the tetanic volley. Between the two peaks, ST decreases similarly to Ca²⁺ concentration time course. This oscillatory behavior is maintained for all the successive stimuli with no significant changes of ST peaks (data not shown). Thus maximum ST is first reached when active tension is ~2% of the peak value of ST was about six times greater than passive response, which indicates that stimulation increases passive stiffness of the fiber by seven times (mean increase in 10 experiments was 7 ± 1). The value of the 3-ms delay to reach the maximum ST is shorter than reported previously in the FDB fibers (6); this is likely due to the higher temperature used here (30°C compared with 24°C).

Effects of sarcomere length on ST. Measurements of passive, active, and ST were made in NT solution at a sarcomere length between 2.6 and 4.4 μm, beyond the theoretical zero overlap length. No measurements were made above 4.4 μm because the bundles were usually unable to recover the original active tension when returned to the initial sarcomere length. To further reduce the number of attached cross bridges, experiments were also made in 10 μM BTS-Tyrode solution, which reduced average force at 2.7 μm by ~76% with respect to NT solution. To compare different fiber responses, ST was expressed relative to \( P_0 \), for 1% \( l_0 \) stretch amplitude [static stiffness (SS)]. The results are shown in Fig. 4A. The sarcomere-length-SS curve in BTS, apart from a slight shift to the right, is almost superimposed to the curve in NT. In contrast, active tension in BTS is ~3.5 times smaller than in NT at any sarcomere length (Fig. 4B). Note that the sarcomere-length-tension curves differ from the expectation of the overlap between myofilaments, which has a maximum at ~2.6–2.7 μm. This is because the tension plotted is not measured at tetanus plateau but on the tetanus rise, 3 ms after the stimulus. Under these conditions, in addition to myofilament overlap, force generation is also modulated by changes in myofibrillar Ca²⁺ sensitivity with sarcomere length (49).

Active tension, in both solutions, had a maximum at ~3.3 μm and fell to zero at ~4-μm sarcomere length. SS increased progressively from 2.6 μm up to a maximum value at 3.4–3.5 μm and then started to decrease again. The sarcomere length at which SS reached zero was not directly measured, but it can be reasonably extrapolated at about 4.5 μm, well above the zero overlap length. At sarcomere length of 4 μm, at which the active tension was near zero, SS was still ~50% of the

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Fig. 2. ST is already present during the latent period, 1 ms after the start of stimulation. Fiber force responses to a stretch applied 1 and 3 ms after the stimulus on the rise of a short tetanus; trace a: fiber length change; trace b: isometric force with stretch; trace c: passive response to the stretch; trace d: subtracted traces showing ST. Isometric responses are omitted for clarity. Note that ST is already present 1 ms after the stimulus when the fiber is developing zero active force. To superimpose the 2 responses, the timing of the stretch was fixed and the stimuli were applied 1 or 3 ms before the stretch. Stretch amplitude = 3.1%; \( l_0 \); duration = 0.63 ms. Sarcomere length = 2.65 μm. Horizontal lines represent zero tension, and vertical dashed line is the change from 1-ms to 10-μs sampling time.

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Fig. 3. Time course of ST is not correlated with active tension. Active tension (○) and ST (●) time course during the first 2 consecutive stimuli of a short tetanus. Stretches were applied at various delay up to 16 ms after the start of stimulation. Zero time is the time of stimulus application. The first ST peak occurred between 2 and 3 ms after the stimulus when tension is very low. The second is reached at a 12-ms delay that is between 2 and 3 ms after the 2nd stimulus. Stimulation frequency = 100 Hz; stretch amplitude = 3.1% \( l_0 \); sarcomere length = 2.63 μm.
maximum, confirming its non-cross-bridge nature. At 2.7 μm, mean SS corresponded to ~3% of P₀ and increased to the maximum value of 5% P₀ at 3.5 μm to decrease to zero at ~4.5 μm. These results do not confirm the finding that force response by stretching in activated myofibrils continues to increase progressively at sarcomere length well above 4.5 μm (29).

**Passive tension.** The sarcomere length-passive tension relation was measured in four bundles, and the result is shown in Fig. 5. The passive tension of the bundle (squares) is not different from that of single fiber (circles) reported previously (6). Comparison with the data in literature shows that curves in Fig. 5 are also similar to the passive curve measured in myofibrils (29). This means that passive stretch responses of our preparation are not influenced by the connective tissue and are attributable mostly to titin filament stretching.

**DISCUSSION**

It is now widely accepted that the steady-state force following the stretch of an activated muscle fiber is greater than the isometric tension developed at the stretched length (10, 11). This force potentiation, known as RFE, has been the subject of large number of investigations, but its mechanism is still debated (13). A force enhancement after stretch, called ST, was demonstrated by our group in frog and mouse single intact muscle fibers after the application of small and fast stretches at low tension on the tetanus rise or at tetanus plateau when force generation was inhibited by BDM (2–4, 6).

**Equivalence between ST and RFE.** A number of findings indicate that RFE and ST are equivalent, both measuring the excess of steady tension that follows the stretch of an active fiber. RFE and ST, in fact, were shown to 1) depend linearly on stretch amplitude, 2) to be independent of the stretching velocity, 3) to increase with sarcomere length, and 4) to last for the whole stimulation period (2, 10, 11). Thus the difference regards mainly the way in which the force potentiation is investigated: fast stretches applied at low active tension in ST measurements and slow stretches applied at tetanus plateau in RFE measurements. The use of fast stretches at low active tension drastically reduced the influence of cross bridges on the force response and the time to the attainment of the steady ST after the stretch (2, 4, 6). ST and RFE are also quantitatively equivalent (8–11). The value of 1.6% P₀ for a stretch of 1% l₀ in frog fibers for RFE reported by Edman et al. (10) is very similar to the values found for ST in frog (2) and consistent with the value of 3% P₀ found here at higher temperature in mammalian muscle.

**Independence of ST from cross bridges.** The numerous findings showing that ST is Ca²⁺ dependent and it is not arising from cross bridges, either force or nonforce generating, have been discussed in previous articles from our group on frog muscle fibers (2–4). Our previous findings and conclusions are fully confirmed here in mouse fibers at near physiological temperature by the observations that in no conditions active tension and ST were correlated to each other. In addition, the new findings that 1) the sarcomere length-SS relation is very similar in NT and BTS solutions in spite of the much smaller active tension developed in BTS and, most importantly, 2) ST is present at sarcomere length beyond myofilament overlap where cross-bridge presence is not expected, give a further
strong support to the hypothesis of the non-cross-bridge nature of ST.

Length-tension relation. The results of Fig. 4 show that SS increases with sarcomere length from 2.7 μm up to a peak at 3.5 μm, decreases again at higher lengths, and reaches zero ~4.5 μm in both NT and in presence of BTS, in agreement with RFE in frog muscle (11). This behavior does not confirm the finding, reported recently, that passive force in activated rabbit myofibrils progressively increases with sarcomere length beyond the zero overlap length up to 6 μm (29). It should be pointed out, however, that given the very different conditions and procedures these experiments are not directly comparable with ours. Apart from the different preparation, there are two important differences: 1) myoplasmic Ca\(^{2+}\) concentration during a just fused tetanus in intact fibers is not constant but oscillates largely synchronously with the stimuli. In myofibrils, Ca\(^{2+}\) concentration is instead constant and over saturating during the whole contraction; and 2) in our case stretches were not >4% \(l_0\) were fast (stretch time of 0.6 ms), and were applied to fiber previously elongated passively to the desired sarcomere length. On the contrary, the experiments on myofibrils employed just one very slow stretch (stretch time of several seconds) of great amplitude (~250% \(l_0\)) spanning the whole range of sarcomere length. A further difference is that Leonard and Herzog (29) measured passive force in activated myofibrils during the stretch itself rather than in steady conditions after it as we did.

The sarcomere length-SS relation in intact fibers shows the existence of an optimal sarcomere length, similarly to RFE in frog muscle (10). The sarcomere length at which SS is maximal is different from that for force generation that occurs at maximum overlap (2.6–2.7 μm) (6). An optimum sarcomere length does not seem present in experiments on skinned fibers or in myofibrils in which the passive tension in activated preparation seems to increase progressively with sarcomere length (25, 29). The reason of this difference is unclear, one possibility is that interfilamental distance could play a role on the mechanism of force enhancement, for example, by changing titin sensitivity to calcium or by changing the calcium promoted actin-titin interaction (15). Interfilamental distance in fact decreases with sarcomere length in intact preparation (constant volume behavior) whereas in skinned fibers, and presumably in myofibrils, it does not (30).

BTS effects. BTS has been shown to reduce tension and the number of attached cross bridges without affecting calcium release (37). On this basis, we should expect no effect of BTS on SS. In general, data in Fig. 4 confirm this expectation, in agreement with previous data on rat muscle (38); however, BTS seems to shift the sarcomere length-SS relationship to the right, which would explain why SS in BTS at 2.7 μm is smaller than in NT solution. The reason for this shift is unknown, but BTS, in addition to inhibit cross-bridge formation, also reduces the calcium sensitivity of the cross bridges (37). It cannot be excluded that BTS has a similar effect on titin calcium sensitivity or titin actin interaction in a sarcomere length dependent way.

**RFE and ST mechanism.** According to several studies (13, 24, 31, 32), RFE is mainly consequence of sarcomere length nonuniformity developing after the stretch on the descending limb of the length tension relationship. This mechanism could be associated with recruitment of passive elasticity during force development due, for example, to misalignment of adjacent myofibrils (12). Nonuniformity of sarcomere length would lead to fiber regions where myofilament overlap is greater than expected from the experimental sarcomere length producing the excess of tension observed. However, overlap nonuniformity does not seems a likely interpretation of our ST measurements. In fact, the sarcomere length nonuniformity and the development of passive stiffness (myofibril misalignment) upon stimulation both require and are expected to be correlated with the presence of force generating cross bridges. Without cross bridges and active force, no change in overlap and no myofibrils misalignment would occur. This is contrast with our findings showing that ST is not correlated with tension and it is present also at zero active tension either during the latent period or at sarcomere length >4 μm. The conclusion that ST does not arise from sarcomere length nonhomogeneity is consistent with the observation that ST is present even in the plateau region of the sarcomere length-tension relation, where sarcomere length nonuniformity is very small and has no influence on tension generation (2, 26). A further proof is given by the finding that force potentiation is present also in a single sarcomere (28).

When we demonstrated for the first time the presence of ST, we postulated the possibility that Ca\(^{2+}\) could quickly stiffen some unknown sarcomeric structure, which we anticipated could be titin or nebulin (3). Consistently with this idea, Labeit et al. (25) showed that \(1\) recombinant proteins, containing both PEVK repeats and glutamate (E)-rich motifs of titin, become stiffer in presence of calcium; and \(2\) Ca\(^{2+}\) increased the force response to slow stretch, with respect to relaxing conditions, of skinned fibers previously treated with gelsolin to eliminate actin filament and cross-bridge formation. Another mechanism for titin stiffening was identified with the phosphorylation of PEVK segment (19, 21). Both mechanisms seem fast enough to make titin stiffer immediately after calcium release and are therefore compatible with our results. However, these mechanisms were estimated to produce a total titin stiffness increases of ~50% of resting conditions (16). This figure seems too small to account for the mean increase of passive stiffness upon activation of sevenfold found here and, in general, for passive force potentiation during stretching (29). To account for this discrepancy and to explain their results in myofibrils, Leonard and Herzog (29) proposed that \(1\) in the presence of Ca\(^{2+}\) titin binds to actin becoming stiffer, and \(2\) titin-actin interaction is promoted and modulated by the cross-bridge action. Another similar, and more elaborated hypothesis, has been proposed by Nishikawa et al. (33). According to these authors, cross-bridges cycling could induce the rotation of thin filaments and winding of titin upon them so as to induce a great extension of the PEVK region and an increase of its stiffness. To be effective, this mechanism is assumed to be preceded by the calcium-induced binding of the N2 segment of titin to actin to eliminate the high compliance of proximal tandem Ig domains at low tensions (15). However, these hypotheses too require the presence of cross bridges to be effective. Force-generating cross bridges are necessary either to promote the titin-actin interaction (29) or to induce winding of titin upon myofilaments (33). Consequently, the extent of RFE or ST is expected to be directly linked to force developed by the fiber. As pointed out before, this is not the case since our previous and present findings show that force potentiation is not correlated with the
tension developed by the fiber and it is present also when the fiber develops zero tension either during the latent period or at zero myofilament overlap. The absence of correlation between ST (or RFE) and the force developed by the fiber seem to rule out any mechanisms based on cross bridges. Thus force enhancement poststretch is mainly attributable to stretching of an elastic sarcomeric structure, noncorrelated to cross-bridge action, whose stiffness greatly increases upon calcium concentration increase, which could be identified with titin. As pointed out above, stiffening of titin by Ca\(^{2+}\) does not seem great enough to explain the stiffness increase of the fiber upon stimulation (16). There are some aspects, however, that could explain at least partially this discrepancy. The experiments on skinned fiber by Labeit et al. (25), from which the stiffening of titin upon Ca\(^{2+}\) binding was derived, were made in soleus muscle, but soleus has a SS of about 1/5 of extensor digitorum longus and 1/2 of FDB (6, 35), which would reduce the discrepancy. Furthermore, the experiments on skinned fibers and myofibrils both showed that tension in passive and Ca\(^{2+}\)-activated fibers increased monotonically with sarcomere length up to 3.5 μm and beyond in myofibrils. On the contrary, experiments in intact preparations from both frog and mouse muscle showed the presence of an optimum sarcomere length at which ST was maximal (2, 6, 11). This different result could be correlated with the constant volume behavior of intact fibers not present in skinned fibers. When intact fibers are elongated, the lateral separation between myofilament decreases. This effect could influence the interaction between Ca\(^{2+}\) and PEVK titin segments. Another possibility is that stiffening of titin filament increases further with the postulated sarcomere length-dependent interaction with actin raising the active stiffness to the measured level. This possibility, however, seems not consistent with recent studies in skinned psoas fibers (8). It is also possible that the PEVK segment has an optimum elongation for Ca\(^{2+}\) sensitivity, which could be attained at sarcomere length ~3.5 μm, explaining the great increase of titin stiffness with calcium and the effects of sarcomere length on the SS. A further possible contribution to stiffening of titin in activated fibers could arise from the stiffening of the immunoglobulin domain 27 in presence of calcium (9). The increment of about 7 times of the passive stiffness upon stimulation reported here is in rather good agreement with the finding that passive stiffness of titin increases by ~2.5 times upon activation in soleus (33). Considering that ST of soleus is about one-half of that of FBD, this would correspond to an increase of five times in FDB sarcomere stiffness.

In conclusion, the data reported in this article show that stimulation, in addition to promote cross-bridge interaction, increases the passive sarcomere stiffness in a non-cross-bridge Ca\(^{2+}\)-dependent way. This stiffness increase, upon a stretch, gives rise to a constant force level, which adds to active force resulting in a force potentiation that is the residual force enhancement by stretch shown by skeletal muscle. Although not directly demonstrated, all our data indicate that passive sarcomere stiffness increase upon activation is due to a calcium based titin stiffening, possibly associated with a calcium-dependent titin-actin interaction.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: M.N. and M.A.B. performed experiments; M.N., G.C., and B.C. analyzed data; M.N., G.C., M.A.B., and B.C. interpreted results of experiments; M.N. and B.C. prepared figures; G.C. conception and design of research; G.C. and B.C. drafted manuscript; B.C. edited and revised manuscript; B.C. approved final version of manuscript.

REFERENCES
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